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March, 1949

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ACTINOMYCOTIC DERMATITIS IN CROSS MERINO LAMBS

By H. K. LALL, B.Sc., M.R.C.V.S., Assistant Animal Husbandry Commissioner with the Government of India and V. R. RAJAGOPALAN, Dip. Bact. (Vict.), Indian Veterinary Research Institute, Mukteswar-Kumaon

(Received for publication on 4 November 1948)

(With plates I to III)

A PECULIAR form of dermatitis was noticed among the lambs at the Government Livestock Farm, Hissar, and, at one time, it caused some concern as mortality among the affected ones was rather high. The disease was observed in 1942 among a few lambs but it attracted particular attention when it assumed serious proportion in 1944. The disease at first, occurred in one of the blocks of the farm and spread to another one, about four miles away, in about two weeks.

Clinical features

The outbreaks occurred in the rainy months of July, August and September and higher incidence was recorded, in pens which were damp, being in low lying areas. The Hissardale—a breed evolved as the result of crossing Bikaneri with Merino, possessing finer wool suffered more than the Lohis or Bikaneris.

Out of 268 Hissardale lambs 91 (34 per cent) got the disease and 26 (28.6 per cent) of the affected animals died. The disease among lambs of the Lohi breed was relatively mild; 21 out of 170 lambs (12.36 per cent) were affected but only one (4.8 per cent) died. No cases were recorded among the Bikaneri lambs. The disease was confined to very young suckling lambs not above a fortnight in age. No adult sheep contracted the disease.

SYMPTOMS AND COURSE

The very early cases usually passed unnoticed. With the progress of the disease, symptoms were more pronounced and the affected lambs showed dullness and depression. The affected portion of the skin thickened and was hard to feel. The parts usually involved were the skin of the back and the neck although sometimes, scale-like lesions also developed around the eyes, nose, lips and ears. The skin of the abdomen, inside of the legs and below the fetlock, was never affected. The skin of the back was often extensively involved and moulded itself into a sort of hard, rigid, inflexible, inelastic plate, the patient then becoming imprisoned in a kind of plaster of Paris jacket. Because of its peculiar disposition, the skin of the neck was wrinkled (Plate I, fig. 2) and the rigidity of the neck interfered with suckling. The wrinkled skin sometime cracked and wounds developed which invariably got infested with maggots, if left untreated (Plate I, fig. 1). The skin on the back lost all wool and presented a raw appearance. In a number of cases, nodules exuding yellowish pus could be seen (Plate II, fig. 1). In recovered cases,

it took a long time, for the skin to assume normality and the wool remained discoloured for quite sometime. Some of the scabs took longer to fall off and with the new wool growing under them, they looked like an odd crop of mushrooms (Plate II, fig. 2). The crop of wool after recovery was got analyzed, as regards crimp, length, elasticity and tensile strength and was found to be normal. It, therefore, appears, there is no permanent damage to the wool follicles.

There was no itching in the initial stages, but in later stages some animals were seen scratching and responded to the scratch reflex.

The course varied from four to fifteen days. The appetite was maintained in those cases in which there was no rigidity of the neck. There was a rise of temperature of one or two degrees in fatal cases, usually on alternate days.

PATHOLOGY

Post-mortem lesions

The skin was hard, congested, and about ten times thicker than the normal. In some cases there were small depressions at the surface of the skin and in others nodular eruptions, but in the majority of cases, however, the skin was like a sheet of parchment. The cut surface, showed the presence of minute pustules (Plate II, fig. 1). The blood vessels of the skin were extremely congested. No lesions, directly referable to the main disease, were detected in any of the internal organs.

HISTOPATHOLOGY

The lesions are referable to a local infectious disease of the skin with the seat of the germ in the epidermis. There is an inflammatory change of the cutis at the epithelial margin, leading to rapid proliferation of the cells of the germinal layer with cornification or parakeratosis and hyperplasia of the prickle cells (Plate III, figs. 1 and 2). The cells of the parakeratotic transitional layer preserve their nuclei which are flattened and in section appear lentil-shaped. The cells of the superficial layers are devoid of nuclei and stain poorly and uniformly. These parakeratotic cells, on account of the pressure of the cornified tissue from above and the strong degree of resistance of the wavy papillary body from below, are thrown into ridges and taps and look under low power as a series of pillars composed of arched cells. There are focal accumulations of polymorphonuclear leucocytes, usually at the base of the arches at their junction with the prickle cells.

The blood vessels of the dermis are congested and those of the papillae are laden with a large number of leucocytes. In some cases the wool follicles are fewer in number. The sebaceous glands are compressed and are not easily distinguishable in grossly affected places.

The collagen fibres of the hypodermis are very thick and numerous as compared with the normal and the blood vessels are congested and contain many polymorphs.

The causal organism is rod-shaped with infrequent branching. In sections they appear of varying thickness. By gram weight they are Gram positive and appear to stain uniformly but under higher power, most of these rods comprise of pairs of biscuit-shaped discs. These discs apparently stain more intensely along the



FIG. 1. Wounds showing raw surfaces

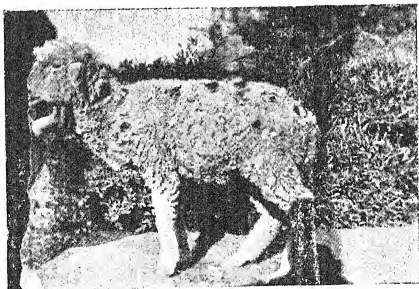


FIG. 2. Showing hardening of the skin and wrinkles

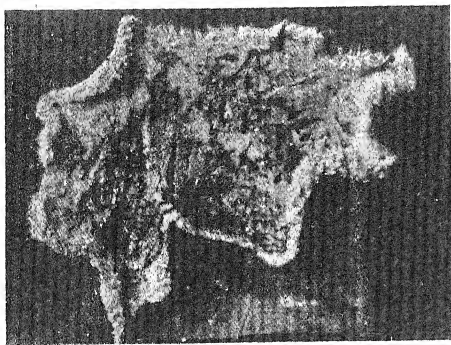


FIG. 1. Inside of the skin showing pitting and pus nodules



FIG. 2. A lamb four months after recovery showing lumpy wool

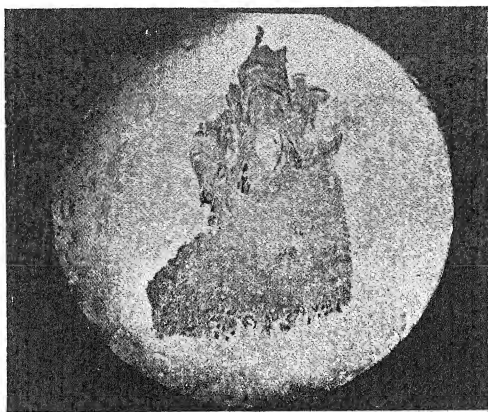


FIG. 1. Hyper keratosis of the epidermis

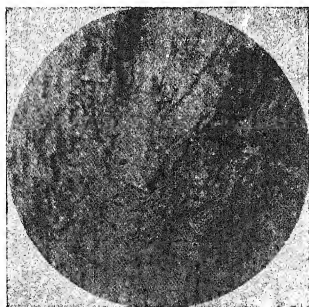


FIG. 2a Mycelia in the epidermis

margin and so appear sometimes as bipolar rods placed side by side in series. At the surface of the skin, these rods break up into coccoid elements. The organisms are distributed in largest numbers at or near the surface of the skin. They work their way up along shaft of the wool follicles and sometimes reach as far down as the papillae of the hair. They are also found working into the epidermis in tortuous courses along the poles of the sweat glands. Sometimes fragments are found distributed throughout the cornified tissues but they never apparently reach the prickle cells or the dermis.

The peculiar distribution of the parasite suggests that they are normally present on the surface of the skin and in the hot rainy months when there is a large outpour of sweat, the stratum corneum becomes soft and affords the parasite a suitable site for its growth and multiplication and the infection probably extends along the sweat pores but the inflammatory changes induced at the stratum germinativum and the attraction of leucocytes through the intercellular lymph spaces of the prickle cells is a distant action possibly effected through the agency of a diffusible toxin.

BACTERIOLOGY

The causal organism which was clearly a mycelium in morphology, could not be cultivated as the lesions, in each case, had been previously treated with preparations containing iodine. Detailed work on the bacteriology of the organism from other outbreaks is under investigation and will be published on completion of the work in due course.

DISCUSSION

From the available literature it appears, that this condition has not been previously recorded in India. Seddon [1927-28] described a form of disease very similar to what we observed except that in our cases the matting of the wool was not a prominent feature. Bull [1922] isolated a mycelium from such cases which he named *Actinomyces dermatonomus*. Morais [1922] described apparently the same disease under the term 'Lumpy wool' and he associated the disease with an excess of moisture owing to continued rainfall. Cases also were marked by 'Lumpy wool'. Morais [1930] was unable to transmit the disease by contact or by scarification, but Fethers [1939] was readily able to transmit the disease.

Among those who have described similar diseases in other species is Albiston [1933] who reported the disease in calves. The organism isolated conformed to the general description of *A. dermatonomus* except that colonies of his cultures were greyish-white in contrast to the yellow colour typical of the type species. Stableforth [1937] and Edgar and Keast [1940] encountered similar conditions in the horse caused by the same organism. The lesion started at the lip and spread to other places. The disease has been reported in the goat by Beaton [1932] in which lesions were found in the abdomen and the inner surface of the forelegs and thighs.

SUMMARY

A form of dermatitis in Hissardale lambs (Bikaneri-Merino crosses) caused by a mycelium is described along with the symptoms, lesions, and histopathology.

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STUDIES ON THE DRYING OF RINDERPEST VIRUS

By N. B. DAS, Indian Veterinary Research Institute, Mukteswar

(Received for publication on 6 August 1948)

THIS paper describes experiments on the drying of rinderpest virus so as to conserve its virulence. The undried virus does not retain the virulence for very long in the Indian field conditions unless maintained on ice or otherwise suitably refrigerated. The dried virus, on the other hand, can resist better the environmental factors, chiefly climatic, which are responsible for the rapid deterioration of the undried product.

Pfaff [1938] dried goat spleen vaccine *in vacuo* over calcium chloride and showed that the dried vaccine was equal in its immunizing effect in cattle and buffaloes to the untreated fresh spleen vaccine. Nair *et al.* [1942] also reported similar results. Robles and Generoso [1934] prepared a vaccine by slow drying of spleen and lymph glands of rinderpest infected animals over calcium chloride in the cold. We have desiccated the virulent virus according to Pfaff as well as by other methods and have also studied the stability of the dried product at such temperature ranges as would be experienced in the transit of dried virus from the laboratory to the field.

EXPERIMENTAL

The virus contained in leucocytes as well as in spleen tissues has been desiccated by different methods. The dried products were tested on hill bulls (Kumauni cattle) for virulence at varying intervals after preparation to study the survival of the virus under certain methods of drying and when exposed to different temperatures for varying periods.

Experiments with leucocyte-cream

Blood of a hill bull, inoculated with rinderpest virus (E-line of this Institute) was collected in sterile flasks containing sodium oxalate (0.2 per cent final concentration). The oxalated blood was centrifuged to separate plasma and the white cells were carefully collected with a pipette. The white cells were freed from the contaminated R. B. C. by repeatedly centrifuging the cell suspension in normal saline. One c.c. of this cream was kept at 4°C as control; 0.1 c.c. of this control sample was tested on a hill bull along with the other samples described below.

Drying on filter paper

A piece of Whatman filter paper (No. 40; 2 sq. c.m.) was soaked with 1 c.c. of the above leucocyte-cream and was dried in a vacuum desiccator over calcium chloride, the desiccator being in an ice-salt mixture for the first few hours and then at 0°C overnight. The evacuation was repeated a few times during the first five hours in order to maintain a high vacuum. Ultimately the dried material was ground in an agate mortar with 2 c.c. normal saline and the whole suspension was injected subcutaneously into a hill bull (Table I).

Drying by cold acetone

One c.c. of the leucocyte-cream was mixed with 5 c.c. of acetone (cooled in ice-salt mixture for two hours prior to use) in a small test tube and was left in ice-salt mixture for five minutes with occasional shaking. The whole precipitate was then collected on a small filter paper (Whatman No. 40) in the refrigerator. The precipitate, along with the filter paper, was dried in a vacuum desiccator over calcium chloride as described above. The dried material was ground with 2 c.c. normal saline and was inoculated into a hill bull (Table I).

Drying by cold alcohol

One c.c. of the leucocyte-cream was mixed as above with 5 c.c. of cold absolute alcohol and was kept in ice-salt mixture for five minutes before filtration. The residue on the filter paper (Whatman No. 40) was dried as above. The dried material was similarly ground with 2 c.c. normal saline and was injected into a hill bull (Table I).

Arrangement of animals, inoculation and recording of temperature, etc.

All the experimental animals described here were kept in alternate stalls in disinfected sheds in order to prevent spread of infection from one to the other. Separate syringes were used for injecting different animals and the operator disinfected his hands after every injection. The control animals, inoculated with untreated virus, were isolated from the experimental animals by accommodating them in a different shed and their temperature was always taken last of all. The thermometers were sterilized by washing first with alcohol and then with carbolic acid solution before recording the temperature of each animal.

TABLE I

Comparative rate of inactivation of leucocyte-virus dried by different methods

Method of drying	Reaction	Survival days
Fresh (untreated)	Good	10
Dried on filter paper	Good	9
Acetone dried	Good	15
Alcohol dried	Delayed	17

From the above Table it appears that the leucocyte-cream virus was not completely destroyed either by drying on filter paper or by precipitation with cold acetone. The delayed reaction in case of alcohol dried virus appears to be due to the changed properties of the virus.

The above results prompted us to study if virulent rinderpest spleen virus could be dried without loss of potency?

Experiments with spleen tissue

The spleen was collected from hill bulls on the 5th day of inoculation with the E-line virus in a sterile vessel covered with ice and was minced twice in a cool and sterile mincer. The resulting spleen pulp was dried by the methods described below.

Drying by cold acetone

The spleen pulp was agitated with five volumes of cold acetone (cooled in ice-salt mixture) for three minutes and was filtered over Buchner funnel under suction. The residue was treated again as described above and was finally kept in a sterile beaker *in vacuo* over calcium chloride in order to get rid of the last traces of moisture, the desiccator, covered with a black cloth, being in ice-salt mixture for two hours and then at 0°C.

After 40 hours the dried tissue weighing 1.1 gm. (equivalent to 5 gm. fresh tissue) was emulsified in 15 c.c. sterile broth and was inoculated subcutaneously into a hill bull. The other half of the dried tissue was kept in a sealed tube at room temperature and was tested after one month (Table II).

Drying by cold acetone and ether

The pulp was treated twice as described above with cold acetone and then with twice its volume of cold ether on a Buchner funnel. The tissues which were almost dry were finally kept, as above, over calcium chloride.

After 40 hours the dried tissue, equivalent to 5 gm. fresh tissue, was injected into a hill bull as mentioned earlier and the other half was kept at room temperature to be tested after a month's storage (Table II).

Drying by cold acetone in the presence of phosphate

The pulp was mixed with a solid phosphate mixture yielding pH 7.0 and was treated with ten volumes of cold acetone for five minutes before filtration under suction. The fine dry powder was then kept over calcium chloride as above.

After 40 hours the dried tissue, equivalent to 5 gm. fresh tissue, was injected into a hill bull and the other half was stored at room temperature (Table II).

TABLE II

Potency of virus dried by different methods and stored for one month at room temperature (about 12°C)

Method of drying	First test		Second test after a month	
	Reaction	Survival days	Reaction	Survival days
Cold acetone	Good	15	Good	11
Cold acetone and ether	Good	10	Good	13
Mixed with phosphate and treated with acetone .	Good	10	Moderate	13

From Table II it appears that spleen virus is not completely inactivated by treatment with cold acetone under different conditions.

It was then proposed to determine the comparative rate of inactivation of the virus dried by different methods as described above. Tables III, IV and V give the figures when the virus is dried by cold acetone, cold acetone followed by ether and cold acetone compared to that dried *in vacuo* over calcium chloride respectively. At least two hill bulls were used for each test.

TABLE III

Comparative rate of inactivation of virus dried by cold acetone

Quantity of tissue inoculated. (Equivalent of fresh tissue)	Dried tissue virus		Fresh tissue virus	
	Reaction	Average survival days	Reaction	Average survival days
1.0 gm.	Good	11	Good	12
0.01 gm.	Good	14	Good	11
0.0001 gm.	Delayed	21	Good	10

Cold acetone dried virus appears to be active even in 0.01 gm. dose while in 0.0001 gm. dose the activity appears to be doubtful. The delayed reaction followed by death may be due to the changed properties of the virus.

TABLE IV

Comparative rate of inactivation of virus dried by cold acetone and ether

Quantity of tissue inoculated. (Equivalent of fresh tissue)	Dried tissue virus		Fresh tissue virus	
	Reaction	Average survival days	Reaction	Average survival days
1.0 gm.	Moderate	15	Good	8
0.01 gm.	Delayed	17	Good	14

Cold acetone and ether appear to have inactivated the virus to a considerable extent.

Drying in vacuo over calcium chloride

The pulp was uniformly spread over a sterile dish in a thin layer and was kept in a vacuum desiccator with calcium chloride, the desiccator being in an ice-salt mixture during the process of evacuation and then in the refrigerator.

The dried tissue was then ground to a fine powder and was emulsified in normal saline immediately before inoculation (Tables V and VI).

TABLE V

Comparative rate of inactivation of virus dried with cold acetone and in vacuo over calcium chloride

Quantity of tissue inoculated. (Equivalent of fresh tissue)	Acetone dried		Calcium chloride dried		Fresh tissue virus	
	Reaction	Average survival days	Reaction	Average survival days	Reaction	Average survival days
1.0 gm.	Good	9	Good	10	Good	11
0.01 gm.	Delayed	15	Good	12	Good	9

From Table V, calcium chloride dried virus appears to be better than acetone dried virus. This lot of acetone dried virus did not react in 0.01 gm. dose as described in Table III.

Attempts were then made to determine the comparative rate of deterioration on storage of the virus dried by cold acetone as well as *in vacuo* over calcium chloride when inoculated in doses equivalent to 1.0 gm. fresh tissue (Table VI).

TABLE VI

Comparative rate of deterioration of dried virus at different temperatures

Temperature of storage	Period of storage	Acetone dried		Calcium chloride dried	
		Reaction	Average survival days	Reaction	Average survival days
Freshly dried	Good	9	Good	10
4°C	2 months	Good	11	Good	9
4°C	4 months	<i>Nil</i>	No death	Good	13
28°C. Being originally at 4°C for 4 months.	7 days	Good	10
37°C	15 days	Good	10
Room temperature (about 12°C) .	3 months	<i>Nil</i>	No death	<i>Nil</i>	No death

Table VI shows that calcium chloride dried virus is superior to acetone dried virus. Calcium chloride dried virus is active even after fifteen days storage at 37°C and after four months storage at 4°C.

SUMMARY

Rinderpest virus contained in leucocytes can be dried by cold acetone as well as on filter paper *in vacuo* over calcium chloride.

Experiments on the drying of rinderpest virus contained in spleen tissue by cold acetone, cold acetone followed by ether, cold acetone in the presence of phosphate as well as *in vacuo* over calcium chloride are described.

Virus dried *in vacuo* over calcium chloride appears to be the best and is active up to 0.01 gm. dose while virus dried by cold acetone was sometimes found inactive in that dose.

Virus dried over calcium chloride has been found potent after at least fifteen days storage at 37°C and four months storage at 4°C. It is not inactivated on storage for seven days at 28°C after being kept at 4°C for four months.

Acetone dried virus was found potent after two months storage at 4°C.

This work was carried out under the guidance of Mr J. R. Haddow.

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SOME EFFECTS OF FEEDING WHITE RATS WITH *RATANJOT* (*ONASMA ECHIOIDES*) IN *VANASPATI* AND ITS PATHOLOGICAL SIGNIFICANCE.

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THE powdered leaves and roots of the plant *Onasma echiodies* Linn. (ratanjot) are in common use in ayurvedic medicine for the treatment of various affections in the human subject and although much has been learned of the therapeutic use of this drug, little is known of its activity in the body after absorption. It has been suggested that the dye extracted from the roots of the plant would be a suitable agent for colouring *vanaspati* to distinguish it from *ghee* when retailed for sale and, in furtherance of this aim, it was considered desirable to test its toxicity on small animals. In studies carried out at this Institute [Pande and others] results have shown the coloured *vanaspati* to be non-toxic when fed to rabbits, as no symptoms of toxicity developed. It was, however, considered advisable to reinvestigate this problem in order to determine if the same factors had any influence on white rats, and whether the addition of the dye to *vanaspati* rendered it toxic by its repeated and continuous absorption in the tissues of the body over a prolonged period and if so, whether it was sufficient to produce symptoms of clinical importance. White rats were chosen for this study, as these animals are mostly used for dietetic experiments on account of their adaptability to a variety of diets; besides in feeding habits they approximate most closely to man in subsisting on foods compounded from different sources. In an attempt to use rabbits for our feeding experiments in the early stages of this work it was found that when *ratanjot* was mixed with *vanaspati* and added to the diet, the feed was totally refused, or eaten very sparingly. Feeding with the aid of a graduated pipette was then resorted to, but here, there was too much resistance on the part of the animal, and the danger of the food entering the trachea and bronchii was always considered a risk, besides a good deal of wastage occurred making it impossible to estimate accurately the total quantity taken. An appreciation of these facts led us to abandon the use of rabbits, as bad results are likely to follow when food unfit for a particular type of animal is forcibly given. A distaste for the coloured *vanaspati* or as a matter of fact for *vanaspati* alone when fed by drenching showed itself by the animal eating very sparingly and often starving itself, thus affording an excellent example of the bad results which follow the abuse of natural laws in connection with dietetics. On the other hand the feeding of rats with coloured *vanaspati* and *vanaspati* alone is quite a simple affair, the rats accustom themselves gradually to this diet and no interference with the normal process of digestion is brought about.

METHOD

Concentrated *ratanjot* in powdered form was obtained from the Director, Indian Dairy Research Institute, Bangalore. A solution of the dye in *vanaspathi* was prepared by gently heating the mixture repeatedly till as much of it as possible was dissolved. This was filtered through cotton wool and the concentrated dye used to colour the *vanaspathi* to produce colour readings of 5, 12, and 25 red units in a half centimeter cell in the Lovibond tintometer. The experiment was carried out on eight rats and two control rats, all within the 85 to 90 days age limit. The rats were divided into four groups and all were kept on the same stock diet consisting of wheat flour 65 per cent, maize 15 per cent, casein 10 per cent, wheat bran 3 per cent, salt 1 per cent, yeast 5 per cent and calcium carbonate 1 per cent, plus an allowance of milk and green daily. In addition, raw goat liver was given twice weekly. The progress of each animal was watched and each was weighed every ten days. Measured quantities of coloured *vanaspathi* calculated at the rate of 1.25 c.c. per 100 gram body weight was given orally once daily to each rat with the aid of a graduated pipette as follows:

Group 1.—Two rats with a solution of the dye in *vanaspathi* with a colour reading of 5 red units and heated to 160-170°C.

Group 2.—Two rats with a solution of the dye in *vanaspathi* with a colour reading of 12 red units and heated to 160-170°C.

Group 3.—Two rats with a solution of the dye in *vanaspathi* with a colour reading of 25 red units and heated to 160-170°C.

Group 4.—Two rats with a solution of *vanaspathi* alone heated to 160-170°C.

At four months from the commencement of the experiment the animals were killed and post-mortems conducted.

CLINICAL HISTORY

In general, it may be said that the symptoms which develop are the outcome of a rich generous diet which in no way impaired the condition of the animal. The large intake of *vanaspathi* in any form was followed by a depressed appetite, marked decrease in activity and a greater laying on of adipose tissue. The animals grew well and the weight increase was progressive till the end of the experiment. No appreciable differences in weight and normal body size of these experimental animals as compared with other rats of the same age groups in our rat colony could be detected. It will be noticed that no significant symptoms were obtained, although slight toxicity might easily exist in the dye without recognition, so that these cases are more of interest to the pathologist than to the clinician.

POST-MORTEM FINDINGS

There was a great increase of fat in the subcutaneous connective tissue and in the folds of the mesenteries of rats receiving the coloured *vanaspathi* and *vanaspathi* alone. The kidneys of all the rats were covered over with fat, but no alterations in naked eye appearance were seen. In some of the rats of Groups 1 to 4, the lungs were seen to contain small nodules which varied in size and number, at times

coalescing to form a small mass. On microscopic examination Gram negative cocciform bacilli were seen which confirmed the identity of the disease to that caused by *Bacterium pseudotuberculosis rodentium*. The most striking feature at post-mortem was that in the livers of rats receiving the coloured *vanaspati*, the surface was studded with haemorrhagic spots giving it a spotted appearance. These lesions were seen to extend to the parenchyma. Varying degrees of differentiation were observed in the livers of those receiving more of the dye, which also showed a wider range of changes on microscopic examination. Macroscopically, the livers on cross section showed a deeper reddening of the hepatic parenchyma with dilated central and peripheral veins. All other organs were normal.

HISTOLOGICAL FINDINGS

A histological examination of the tissues of all rats under the experiment was carried out. The organs examined were the heart, lungs, liver, spleen, kidney, stomach, intestines, and eye. The examination showed very few changes in the tissues examined. Slight changes were found in the spleens of rats receiving larger amounts of the dye which showed congestion and dilatation of the venous sinuses. Patches of broncho-pneumonic lesions were frequently found in some of the lungs indicating a secondary infection, but as these lesions had no relation whatsoever with the experiment itself, they did not merit further consideration. The main changes were in the liver of rats receiving the coloured *vanaspati*, details of which are given below.

Rats in Group 1

A slight widening of the hepatic capillaries with no changes in the liver cells.

Rats in Group 2

Hepatic capillaries engorged. Liver cells are seen to disintegrate, fade and gradually disappear, leaving pale areas devoid of liver cells.

Rats in Group 3

The hepatic capillaries are more deeply engorged. The liver substance is congested, although the inflammatory process is not evenly distributed. Here too the liver cells are seen to disintegrate and fade, leaving in some parts of the hepatic tissue pale areas completely devoid of liver cells.

DISCUSSION

It will be seen from the above that the most important changes are brought about in the liver, following the presence of an irritant in the portal blood. It is an interesting fact that the absorption of the dye after a period of feeding for four months does not cause any special symptoms, although the morbid change affects the liver of the animals in varying degrees. Further, it does appear entirely improbable that the presence of these lesions in the bodies of the rats should be accidental, especially since the gradations of the lesions in the three groups of rats vary in severity with the amount of the dye fed.

SUMMARY

The feeding of *ratanjot* dissolved in *vanaspathi* (with a colour reading of 5 red units in a half centimeter cell under Lovibond tintometer and heated to a temperature of 160-170°C) for a period of four months did not reveal any toxic symptoms, but on autopsy hemorrhagic spots were present under the capsule of the liver which might also be seen in the parenchyma. No changes in the liver cells were observed.

In the case of rats of groups 2 and 3, which were fed continually for a period of four months with a higher concentration of the drug having a tintometer reading of 12 and 25 red units, the livers showed definite changes, with severe destruction of liver cells. Based on the evidence obtained from feeding rats with *ratanjot* dissolved in *vanaspathi* with a colour reading of 5 red units, it would appear to be advisable to use a much lower concentration of the dye to tint vegetable fats to avoid the harmful effects on the liver, if it is desired to use this substance as a colouring agent. No abnormal changes were observed in the livers of the control rats.

ACKNOWLEDGEMENT

We wish to express our thanks to Dr N. D. Kehar for his great interest and assistance in this work.

THE RELATIONSHIP BETWEEN THE DISTRIBUTION OF THE OX WARBLE-FLY (*HYPODERMA LINEATUM* DE VILLERS) AND SOIL MOISTURE IN INDIA*

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THE outstanding fact in the regional distribution of warble flies is their occurrence in dry, arid localities and their rarity in, or almost complete absence from, moist areas. Thus, Bishop and his collaborators [1926] working in the States of America, state that warble grubs are not found in cattle in low swampy areas; whilst, according to Smith [1932], they are more numerous in Kansas after a dry spring than after a wet one. Walton [1925], in his account of warble-fly infestation among cattle in Great Britain observes: 'Tabulated records suggest a diminution in the abundance of warble larvae following a cool, rainy spring in the preceding year.' In India, Fletcher and Sen [1930] refer to the infestation being 'as a rule, restricted to North-Western India, and absent from the moist areas, such as Madras, Assam, Burma and Ceylon'. Numerous observations made by the present writer in the course of his warble-fly surveys of different localities in India, point to the same conclusion.

Thus, during the winter of 1938 the writer examined 178 heads of cattle of the Dacca Government Cattle Farm in Bengal and none of these showed warble larvae on the back, in spite of the fact that for some previous years warble-infested bulls had been imported into Dacca from Hissar (Punjab). A similar observation was recorded at Trivandrum in South India. Here the writer examined about 20 dairy cows recently imported from Karachi and all showed fully developed *H. lineatum* larvae in their backs, the infection in these cases having been apparently acquired before importation into Trivandrum; for no tumours were found on local breeds of cattle or on cows previously imported from Karachi. During 1937-38, the writer, in collaboration with Mr S. R. Chadha, carried out a survey of the North-West Frontier Province and the infestation was found to be 'very heavy in the hilly districts.' In places where the land is marshy all along, e.g., in Bannu, the warble-fly is very rare but in the dry sandy areas like Dera Ismail Khan it has been most abundant [Chadha and Soni, 1939]. Likewise, in Rajputana, 5,355 (68.65 per cent) heads of cattle were found infested out of a total of 7,800 examined there by the writer in November 1941, whilst in certain tracts not one animal was found free from the infestation. It is noteworthy that similar observations have also been recorded in the case of the goat warble-fly (*H. crossii*), for Cross and Patel [1921],

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working in the Punjab, observed that the percentage of goats harbouring the larvae of this fly varied from 48.3 in some flocks to 91.8 per cent in others. A very high incidence of warble-fly infestation in goats has also been recorded from the North-West Frontier Province, Sind and Baluchistan.

In as much as the adult and the egg-stage periods of *H. lineatum* are very short and its entire larval life is spent inside the animal's body, atmospheric humidity and temperature can have little influence on these stages of its life-cycle. On the other hand, the pupal period normally lasts for from 4 to 6 weeks and is spent underneath the soil surface. It would, therefore, seem more than probable that one, if not the only, condition that ensures the viability of the fly in its pupal stage is the freedom of the soil from excessive moisture. This is illustrated in the case of the district of Gujranwala in the Punjab, where, inspite of heavy warble infestation in the adjoining areas, the water-logged condition of the soil in certain localities of this district has apparently operated against the development of *H. lineatum* in its pupal stage. A similar example is provided by the marshy tract of *tehsil* Laki in the district of Bannu, North-West Frontier Province. Support to the foregoing hypothesis is also lent by the fact that, whilst varying degrees of infestations have been seen in the hilly districts of Darjeeling, Kalimpong, Nilgiris and Shillong, the pest has been found to be wholly or almost absent from the adjoining plains, this being attributable, inspite of frequently heavy rains in these districts, to the quick loss of the moisture content of the soil due to declivity and gravelly nature of the localities. It is of interest that, at Darjeeling and Kalimpong, the incidence of warble-fly infestation was sometimes as high as 20 to 30 per cent. Bruce [1938], working in the United States of America, has also referred to the influence of soil moisture on pupal development in *H. lineatum*. He observes, 'Cattle grubs, *Hypoderma bovis* De G. and *Hypoderma lineatum* (De Vill.), are found throughout the United States, but there are certain areas where they are scarce or periodically absent. Most of these areas are small and scattered, but there is one large area, the Red River Valley of the North, where, under average climatic conditions, cattle grubs are entirely absent. To most stockmen of this area cattle grubs are unknown. Quite naturally the question arises, what factor or set of factors is responsible for the scarcity of cattle grubs in this area? This question was studied by the Bureau of Entomology from 1929 through 1932. Apparently the dominant controlling factor is excessive soil moisture.'

Observations made on *H. lineatum* larvae and pupae kept under known moisture conditions at the Mukteswar laboratory have shown that grubs placed on soil saturated with moisture died without pupating. Out of a dozen pupae left over for 50 days on soil containing 20 per cent moisture and over, not a single emergence took place, and the pupae on dissection showed no trace of adult development. Also no fly emerged from a batch of twelve pupae kept at 15 per cent soil moisture and on dissection only two of the puparia showed partially developed adults. In a batch of six pupae kept at 10 per cent soil moisture, only one emergence took place, the pupal period in this case being 42 days. Pupae kept in containers with soil moisture varying from 1 to 5 per cent yielded the maximum number of emergences,

for out of a batch of nine pupae thus treated six fully developed adults emerged, the average pupal period in these cases varying from 14 to 18 days. A constant temperature of 22°C. was maintained throughout these experiments. These results are summarized in the following Table I.

TABLE I

Observations made on H. lineatum larvae and pupae kept at known moisture conditions

Percentage of soil moisture	Percentage of emergences	Pupal period
20	nil	..
15	nil	..
10	16.6	42 days
1 to 5	66.6	14 to 18 days

The laboratory observations described above are claimed to demonstrate conclusively the intimate relationship of soil moisture with the pupal development of *H. lineatum* and indirectly with the regional distribution of the pest. It is of interest that they are in accord with the results of some field experiments carried out by Bruce [1938] in the United States of America, for these results 'showed that although *Hypoderma* pupae would not develop in wet soil, the same soils, provided with ample drainage, would permit complete development and adult emergence.'

SUMMARY

An excess of soil moisture has been found to have detrimental effect on the development of the *Hypoderma lineatum* in its pupal stage, which normally extends over a period of nearly six weeks. Experiments carried out in the laboratory at Mukteswar have shown that the maximum number of emergences occur at a soil moisture varying from 1 to 5 per cent. No fly emerged from pupae kept at a soil moisture of 15 per cent and above. It is concluded that, it is the moisture content of the soil that accounts for the concentration of the *H. lineatum* in certain localities and its absence from others. This conclusion is borne out by field observations on the regional distribution of the pest in India and in the United States of America.

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THE NUTRITIVE VALUE OF THE INDIGENOUS GRASSES OF ASSAM

The aquatic grasses, their chemical composition and nutritive value

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THE importance of the uncultivated grasses in the economic feeding of our cattle needs no special emphasis. In the eastern provinces of the country where conditions are favourable, they seem to grow in abundance during their season time. Organised attempts have not been made to utilise these grasses as cattle feeds, partly because little is known about their palatability, chemical composition and nutritive value. However, data on the composition of some of the highland indigenous grasses are available [Sen, 1937] and [Kohar, 1947] but there seems to be no reference in the literature either in regard to the chemical composition or the nutritive value of the aquatic grasses which grow in abundance almost in every part of Assam.

In view of the acute shortage of fodder in the country, it has been considered desirable to explore the possibilities of utilising these stuffs as cattle feeds. We have recently completed some detailed investigations as to whether the aquatic grasses can be profitably utilised in the dietary of our cattle as green fodder, hay and silage. The object of the present communication is to give an account of these investigations as follows :—

- I. The chemical composition with progressive maturity as represented by the monthly cut samples under different soil conditions.
- II. The metabolism of the organic and inorganic nutrients as influenced by feeding the grasses as green fodder, hay and silage.

The aquatic grasses which can be collected in large quantities are of two kinds commonly known as *dal* (*Hymenachne amplexicaulis* Nees) and *uridal* (*Oryza sativa* var. *Fatua*) in this part. The *dal* contains a spongy pith, while the *uridal* is free from it. Even when fully mature, they are leafy, soft and succulent. It has been estimated that the yield of these grasses from a medium size *dalani* (lowlying area) of 500 acres is about three lakh maunds per year.

CHEMICAL COMPOSITION

To study the chemical composition on a monthly cut system, samples of grasses were collected from three different places namely, Khanapara, Tongra and Panjabari in case of *dal*, while *uridal* was collected from Beltola. For the sake of convenience and better control in the method of sampling, etc., these areas which were quite close to our laboratory were selected. The chemical analyses have been done according to A. O. A. C., 1945 edition and Talapatra S. K., Ray S. C., Sen K. C. [1942]. The results are shown in the following Tables.

TABLE I
Protein content of the monthly cut samples on dry basis

Month	Dal grass			Uridal grass
	Khanapara	Tongra	Panjabari	Beltola
April	27.1	28.1	29.4	..
May	15.6	17.8	14.1	16.2
June	14.8	10.8	13.1	13.5
July	14.1	11.5	11.9	14.7
August	14.0	11.0	10.6	12.0
September	13.4	12.2
October	13.8	10.8	13.8	11.8
November	13.1	10.6	10.4	10.5
December	9.3	9.4	10.8	10.2

The data in the above Table will show that the aquatic grasses are rich in protein. Even in December when most of the grasses turn woody and fibrous, the aquatic grasses retain as much protein as can be found in some of the cultivated fodder plants like maize, *jowar*, Napier and Guinea at their early stages.

TABLE II
Calcium content of the monthly cut grasses on dry basis

Month	Dal grass			Uridal grass
	Khanapara	Tongra	Panjabari	Beltola
April	0.332	..	0.240	..
May	0.29	0.240	0.401
June	0.300	0.26	0.280	0.380
July	0.303	0.29	0.271	0.300
August	0.270	0.23	0.270	0.340
September	0.260	0.330
October	0.210	0.24	0.220	0.290
November	0.141	0.27	0.200	0.270
December	0.130	0.20	0.140	0.250

The results in Table II show that there is little variation in the content of calcium between the samples collected from the three different places. Compared to *dal*, the *uridal* is definitely richer in calcium. With the progressive maturity, the content of this mineral decreases. Both the grasses may be regarded as poor in calcium.

TABLE III

Phosphorus content of the monthly cut grasses on dry basis

Month	Dal grass			Uridal grass
	Khanapara	Tongra	Panjabari	Beltola
April	0.294	..	0.441	..
May	0.541	0.490	0.422
June	0.300	0.382	0.341	0.382
July	0.312	0.391	0.331	0.300
August	0.281	0.342	0.292	0.342
September *	..	0.291	0.335
October	0.200	0.351	0.211	0.290
November	0.210	0.260	0.220	0.251
December	0.160	0.210	0.200	0.210

The data shown in the above Table are self-explanatory. The grasses seem to be rich in phosphorus, specially at their early stages. With the progressive maturity, the content of phosphorus also decreases and is lowered by almost half at the flowering stage. Samples were also collected from the various representative tracts of the province and analysed. The results are shown below in Table IV.

TABLE IV

Chemical composition of the grasses from all-over the province on dry basis

Localities			Crude protein		Calcium		Phosphorus	
			<i>Dal grass</i>					
Upper Assam	{	1 . .	15.2	12.8	0.20	0.23	0.28	0.23
		2 . .	14.4		0.30		0.25	
		3 . .	8.3		0.19		0.17	
Central Assam	{	1 . .	12.2	11.5	0.30	0.32	0.35	0.34
		2 . .	11.1		0.37		0.33	
		3 . .	11.2		0.30		0.351	
Lower Assam	{	1 . .	21.9 (pit-soil)	12.9	0.15	0.11	0.16	0.30
		2 . .	11.0		0.11		0.29	
		3 . .	14.9		0.11		0.31	
			<i>Uridal grass</i>					
Upper Assam	1 . .	12.0		0.27		0.21		
Central Assam	2 . .	11.6		0.25		0.22		
Lower Assam	3 . .	12.4		0.19		0.21		

A perusal of the data in Tables I to IV will show that while the content of protein is fairly constant irrespective of the places from which samples were analysed, the content of calcium varied from 0.14 in the case of Lower Assam to 0.32 in central Assam. It may, therefore, be reasonably concluded that—

- (a) the aquatic grasses are fairly rich in protein ;
- (b) they seem to be poor in calcium ;
- (c) they are not deficient in phosphorus.

NUTRITIVE VALUE OF THE AQUATIC GRASSES

Although the aquatic grasses are quite rich in some of the indispensable nutrients like the protein and phosphorus, contrary to expectation, they are not much relished by cattle, specially at the early stages when fed as green fodders. It appears that due to some fishy smell, our cattle refuse these stuffs. Whatever may be the cause, as a result of this practical difficulty, we were about to abandon our plan of investigations, but soon it was realised that at least one of these namely, the *dal* grass could be fed to appetite at the flowering stage as a green fodder. But soon after the trial it was found that if the aquatic grasses were fed green, cattle suffered from worm trouble and that the feeding of these grasses to young animals led to serious consequences.

It was, therefore, finally decided that these should be better utilised in the form of hay or silage. The following experiments were therefore, designed :—

- (a) The grass *dal* as a green fodder at the flowering stage.
- (b) The grass *dal* as a silage and as a hay also at the flowering stage.
- (c) The grass *uridal* as a hay at the pre-flowering stage when the yield is maximum.

(a) *The dal grass as a green fodder*

To ascertain the nutritive value of the *dal* grass at the flowering stage, it was fed to four adult Assamese bullocks. The average body-weight of the animal was about 400 lb. They were fed for a period of about one month with the experimental food. Urine and faeces were then collected for ten days according to the technique followed in the Central Research Institute at Izatnagar. Moisture was determined in the daily grass samples as also in the residues. The chemical composition of the ten day composite sample of the grass fed to the animals is shown below :—

Crude protein	9.38
Ether extract	2.30
Ash	12.20
Organic matter	87.80
Fibre	22.10
N-free extract	54.02
Total carbohydrates	76.12
Calcium (Ca)	0.13
Phosphorus (P)	0.206

It is obvious from the above composition that the grass is rich enough in protein to meet the normal requirements of the adult bullocks at rest. In regard to the minerals, the phosphorus content seems to be adequate to support a bullock at rest, while the content of calcium is definitely low. Yet, no attempt was made to balance the ration with calcium carbonate or chalk, for ultimately it is the availability which matters in the assimilation of this mineral and the total intake is of no consequence. The grass was fed to our animals as a single feed.

TABLE V
Dry matter consumption of the grass dal

Animal Number	Body weight lb.	Dry matter consumed lb.	Dry matter consumed per 100 lb. body weight lb.	Average consumption per 100 lb. body weight lb.
1	439	10.1	2.3	2.6
2	312	8.9	2.9	
3	387	9.1	2.4	
4	441	11.3	2.6	

The average consumption of dry matter was well over 2 lb. per 100 lb. body weight and as such the palatability of the grass at the flowering stage cannot perhaps be questioned.

TABLE VI

Digestibility coefficients of the organic nutrients

Animal Number	Intake gm.	Voided in faeces gm.	Amount digested gm.	Per cent digestibility	Average
<i>Dry matter</i>					
1	4,573	1,883	2,690	58.8	58.6
2	4,067	1,608	2,459	60.4	
3	4,175	1,710	2,465	59.0	
4	5,150	2,254	2,896	56.2	
<i>Crude protein</i>					
1	4,28.0	162.5	266.4	62.1	61.5
2	3,81.5	142.5	239.0	62.6	
3	3,91.6	151.3	240.3	61.4	
4	4,83.1	192.5	290.6	60.0	
<i>Ether extract</i>					
1	105.18	65.91	39.27	37.3	37.9
2	93.54	56.44	37.10	39.6	
3	96.03	61.73	34.30	35.7	
4	118.45	72.13	46.32	39.1	
<i>Crude fibre</i>					
1	1,010.6	412.4	598.2	59.2	60.5
2	898.8	348.9	549.9	61.2	
3	922.7	359.4	563.3	61.0	
4	1,138.2	450.8	687.4	60.4	
<i>N-free extract</i>					
1	2,470.3	799.7	1,670.6	67.6	67.0
2	2,197.0	663.0	1,534.0	69.8	
3	2,255.3	735.6	1,519.7	67.4	
4	2,782.0	1,020.2	1,761.8	63.3	
<i>Total carbohydrates</i>					
1	3,480.0	1,212.1	2,268.0	65.2	65.1
2	3,095.8	1,011.9	2,083.9	67.3	
3	3,178.0	1,095.0	2,083.0	65.5	
4	3,920.2	1,471.0	2,449.2	62.5	

The data in the above Table will show that except the ether extract, the digestibility figures are quite satisfactory. From the average digestibility co-efficients, the starch equivalent has been calculated according to the method of Kellner and is shown below along with some of the important cultivated fodder plants.

	Digestible nutrients in terms of 100 lb. of green stuff	
	D.P.	S.E.
<i>Dal</i>	1.46	12.45
Maize	1.04	11.70
Guinea (Young)	1.11	7.30
Napier	0.96	9.6
Jwar	0.84	7.2
Bajra	1.08	11.9

Calcium phosphorus and nitrogen balances under green dal feeding.—The data shown below in Table VII indicate that a positive retention of either calcium or phosphorus is not assured under *dal* grass feeding. A careful examination of the data will show that the ingestion of both calcium (8.1 gm. CaO) and phosphorus (21.4 gm. P_2O_5) on an average is most favourable for the formation of insoluble tricalcium phosphate. The heavy retention of nitrogen is perhaps purely of a temporary nature.

TABLE VII

Calcium, phosphorus and nitrogen balances under green dal feeding

Animal Number	Intake grams	Voided in faeces grams	Voided in urine grams	Total voided grams	Balance grams
<i>Calcium</i>					
1	5.94	8.30	0.4	8.70	-2.76
2	5.29	6.08	0.5	6.58	-1.29
3	5.43	6.52	0.5	7.02	-1.59
4	6.70	8.18	1.4	9.58	-2.88
<i>Phosphorus</i>					
1	9.42	10.36	0.10	10.46	-1.04
2	8.38	8.63	0.15	8.78	-0.40
3	8.60	8.98	0.07	9.05	-0.45
4	10.61	10.84	0.05	10.89	-2.28
<i>Nitrogen</i>					
1	68.60	26.0	24.13	50.13	+18.47
2	61.00	22.8	21.33	44.13	+16.87
3	62.63	24.2	23.47	47.67	+14.96
4	77.25	30.8	24.96	55.76	+21.49

(b) The grass dal as a hay and silage

While the feeding of the aquatic grasses as green fodders leads to helminthic infection, the question of utilising the stuffs in the form of either hay or silage has become the subject matter of our present studies. In order, therefore, to ascertain the best form in which these grasses should be conserved, a number of metabolism experiments were conducted, the details of which are given below.

Metabolism trials with the conserved dal grass.—Two hundred maunds of the green *dal* at the flowering stage were collected from places round about our laboratory, of which 100 maunds were ensiled and the rest made into hay according to the 'Tripod system', the description of which as also the method of making had formed part of a separate paper. Adult Assamese bullocks having an average body weight of about 400 lb. were used as experimental subjects. The trial with hay was conducted first and the same animals were later on switched on to silage feeding and after the preliminary experimental period, urine and faeces were collected as usual. The chemical composition and the digestibility coefficients of the hay and silage are shown in the following Tables :

TABLE VIII
Chemical composition of the hay and silage on dry basis

	Hay	Silage
Crude protein	7.5	6.94
Ether extract	1.43	1.85
Ash	12.90	17.90
Organic matter	87.10	82.10
Fibre	20.20	27.80
N-free extract	48.97	45.51
Total carbohydrates	78.17	73.31
Calcium (Ca)	0.141	0.175
Phosphorus (P)	0.186	0.094

The data in the above Table show that both the samples are almost identical in chemical makeup. The hay is, however, slightly richer in protein than the silage.

TABLE IX

Digestibility coefficients of the organic nutrients of dal grass when fed as hay and silage

Animal	Number	Intake grams	Voided in faeces grams	Amount digested grams	Per cent digestibility	Average
<i>Dry matter</i>						
Hay feeding	1 .	3,360	1,400	1,960	58.4	56.2
	2 .	3,201	1,428	1,773	55.4	
	3 .	2,869	1,206	1,663	57.3	
	4 .	4,326	2,000	2,326	53.8	
Silage feeding	1 .	4,031	1,625	2,406	59.7	58.5
	2 .	3,473	1,532	1,941	55.9	
	3 .	3,992	1,522	2,470	62.0	
	4 .	5,064	2,197	2,867	56.6	
<i>Crude protein</i>						
Hay feeding	1 .	252.0	148.1	103.9	41.2	42.4
	2 .	240.1	132.5	107.6	44.8	
	3 .	215.2	125.0	90.2	41.8	
	4 .	324.5	189.4	135.1	41.6	
Silage feeding	1 .	279.8	157.1	122.7	43.8	43.9
	2 .	241.0	131.3	109.7	45.5	
	3 .	277.0	149.4	127.6	46.0	
	4 .	351.4	200.4	142.0	40.4	
<i>Ether extract</i>						
Hay feeding	1 .	48.05	28.42	19.63	40.8	39.1
	2 .	45.77	27.13	18.64	40.7	
	3 .	41.03	25.93	15.10	36.7	
	4 .	61.86	38.20	23.66	38.2	
Silage feeding	1 .	74.57	43.88	30.69	41.1	40.9
	2 .	64.25	39.83	24.42	38.0	
	3 .	73.85	42.62	31.23	42.2	
	4 .	93.68	54.05	39.63	42.3	

TABLE IX—*contd.**Digestibility coefficients of the organic nutrients of dal grass when fed as hay and silage*

Animal Number	Intake grams	Voided in faeces grams	Amount digested grams	Per cent digestibility	Average
<i>Crude fibre</i>					
Hay feeding	1 .	981.1	282.1	699.0	71.2
	2 .	934.7	271.3	663.4	70.9
	3 .	837.7	229.4	608.3	72.6
	4 .	1,263.2	404.0	859.2	68.0
Silage feeding	1 .	1,120.6	346.1	774.5	69.1
	2 .	965.5	293.2	672.3	69.6
	3 .	1,109.8	319.9	789.9	71.6
	4 .	1,407.8	454.8	953.0	67.6
<i>N-free extract</i>					
Hay feeding	1 .	1,045.4	602.6	1,042.8	63.3
	2 .	1,567.5	658.6	908.9	57.9
	3 .	1,404.9	529.0	875.9	62.3
	4 .	2,118.4	868.4	1,250.0	59.0
Silage feeding	1 .	1,834.5	683.0	1,151.5	62.7
	2 .	1,580.6	684.7	895.9	56.6
	3 .	1,816.8	669.1	1,147.7	63.1
	4 .	2,304.6	949.1	1,355.5	58.8
<i>Total carbohydrates</i>					
Hay feeding	1 .	2,626.5	884.7	1,741.8	66.3
	2 .	2,502.3	929.9	1,572.3	62.8
	3 .	2,242.6	758.4	1,484.2	66.1
	4 .	3,381.6	1,273.4	2,109.2	62.3
Silage feeding	1 .	2,955.1	1,029.1	1,926.0	65.1
	2 .	2,546.1	977.9	1,568.2	61.5
	3 .	2,926.6	989.0	1,937.6	66.2
	4 .	3,712.4	1,403.9	2,308.5	62.1

The data from the above Table may be summarised as follows :—

	Average digestibility coefficients	
	Hay	Silage
Dry matter	36.2	38.5
Crude protein	42.4	43.9
Ether extract	39.1	40.9
Crude fibre	70.7	69.3
N-free extract	60.6	60.3
Total carbohydrates	64.4	63.7

The above data will show that there is very little difference in the digestibility coefficients of the organic nutrients under the two sets of feeding. From the average digestibility figures, the starch equivalent (Kellner's) has been calculated and is found to be 31.22 and 12.34 in the case of hay and silage respectively.

TABLE X

Calcium, phosphorus and nitrogen balances under hay and silage feeding

Animal Number	Intake grams	Voided in faeces grams	Voided in urine grams	Total voided grams	Balance grams
<i>Calcium</i>					
Hay feeding	1 .	4.74	5.72	0.81	5.90
	2 .	4.51	5.04	0.14	5.18
	3 .	4.03	4.19	0.15	4.34
	4 .	6.10	7.13	0.43	7.56
Silage feeding	1 .	7.05	8.26	0.12	8.38
	2 .	6.08	6.39	0.20	6.56
	3 .	7.00	5.63	0.40	6.03
	4 .	8.86	7.98	1.50	9.48

TABLE X—*contd.**Calcium, phosphorus and nitrogen balances under hay and silage feeding— contd.*

Animal Number	Intake grams	Voided in faeces grams	Voided in urine grams	Total voided grams	Balance grams
<i>Phosphorus</i>					
Hay feeding	1 .	6.25	4.20	0.05	+ 2.00
	2 .	5.95	5.28	0.06	+ 0.59
	3 .	5.33	4.64	0.02	+ 0.67
	4 .	8.05	6.80	0.10	+ 3.15
Silage feeding	1 .	3.79	4.71	0.10	— 1.02
	2 .	3.26	4.23	0.03	— 1.00
	3 .	3.75	3.70	0.08	— 0.03
	4 .	4.76	5.02	0.04	— 0.30
<i>Nitrogen</i>					
Hay feeding	1 .	40.32	23.7	13.74	+ 2.88
	2 .	38.41	21.2	9.30	+ 7.91
	3 .	34.43	20.0	12.94	+ 4.49
	4 .	51.91	30.3	17.10	+ 4.61
Silage feeding	1 .	44.74	25.44	8.47	+ 11.13
	2 .	38.55	21.00	7.87	+ 9.68
	3 .	44.31	23.90	8.60	+ 11.81
	4 .	56.21	33.50	9.32	+ 13.39

It appears from the data shown in the above Table that a positive calcium balance is not assured whether the grass is fed as a green fodder, hay or silage. The positive phosphorus balance under hay feeding is difficult to account for, the only explanation is that the ingestion of this element under hay feeding was almost double than that of silage feeding. A positive nitrogen balance is noticed under both sets of feeding.

It has been found that 100 maunds of the aquatic grass *dal* yield the following quality and quantity of hay and silage at the flowering stage :—

Dwt. grass taken	Conserved product	Quality	Yield	Dry matter per cent	Digestible nutrients	
					D.P.	S.E.
Maunds			Maunds		Maunds	Maunds
100	Hay	Medium	32	84	0.848	0.999
100	Silage	"	75	30.8	0.713	0.926

It is obvious that whichever method is adopted, the results appear to be the same. While silo-making requires a greater technical skill, hay making is simple.

In conclusion it may be stated that the grass can be fed to cattle at the flowering stage as a green fodder, hay or silage. If it is fed as green, the animals are exposed to the danger of helminthic infection. The grass is deficient in calcium.

(c) *The grass uridal as a hay*

It has already been stated that the feeding of the aquatic grasses as green fodders leads to worm trouble, attempts have, therefore, been made to utilise them as hay or silage. Accordingly, large quantities of hay and silage were made from this grass. The silage, however, turned highly acidic and could not be fed to animals up to appetite.

Excellent quality hay was prepared out of this grass by the 'Tripod system'. About 50 per cent of the original green colour of the grass could be retained, even though the hay was nine months old. The results of the metabolism experiment are shown below. It may be mentioned that the hay was fed to three adult bullocks an exclusive feed.

TABLE XI

(Uridal) hay consumption as compared to paddy straw

Animal	Number	Body weight lb.	Total dry matter consumed lb.	Dry matter consumed per 100 lb. (lb.)	Average consumption per 100 lb. (lb.)
Uridal	1	392	10.0	2.6	2.4
	2	380	8.5	2.2	
	3	370	8.5	2.3	
Paddy-straw	5	350	5.3	1.5	1.6
	6	340	5.7	1.7	

Although the grass is not much eaten either as a green fodder or as a silage, the hay consumption, as shown in the above Table, seems to be very satisfactory. It is quite interesting to note that compared to paddy straw, the hay consumption is 50 per cent more.

TABLE XII

Digestibility coefficients of uridal hay

Animal Number	Intake of hay grams	Voided in faeces grams	Amount digested grams	Per cent digestibility	Average
<i>Dry matter</i>					
1	4,530	2,040	2,490	55	55
2	3,860	1,754	2,106	55	
3	3,805	1,700	2,105	55	
<i>Crude protein</i>					
1	371.2	185.0	186.2	50	50
2	316.3	132.5	183.8	52	
3	311.9	162.5	149.4	48	
<i>Ether extract</i>					
1	70.7	61.2	48.5	23	22
2	67.9	53.3	44.6	22	
3	67.0	52.7	44.3	21	
<i>Crude fibre</i>					
1	1,449.6	408.0	1,041.6	72	71
2	1,235.2	326.2	909.0	74	
3	1,217.6	309.4	908.2	75	
<i>N-free extract</i>					
1	1,916.6	838.4	1,078.2	56	55
2	1,633.2	749.0	884.2	54	
3	1,609.9	732.7	877.2	54	
<i>Total carbo- hydrates</i>					
1	3,366.2	1,246.4	2,119.8	63	63
2	2,808.4	1,075.2	1,793.2	62	
3	2,827.5	1,042.4	1,787.4	63	

The digestibility figures for *uridal* hay are quite satisfactory. The starch equivalent as calculated by Kellner's method was found to be 28.12 per cent.

It may be seen from the figures shown below in Table XIII that when made into hay, the grass *uridal* is superior to that of *dal* in so far as the content of digestible protein is concerned.

TABLE XIII

Dal hay as compared to uridal hay as cattle food

Nutrients	Chemical composition		Average digestibility coefficients		Digestible nutrients			
	<i>Dal</i> hay	<i>Uridal</i> hay	<i>Dal</i> hay	<i>Uridal</i> hay	<i>Dal</i> hay		<i>Uridal</i> hay	
					D.P.	S.E.	D.P.	S.E.
Dry matter . . .	84.0	85.0	56	55	2.65			
Crude protein . .	7.5	8.19	42	50		51.2		
Ether extract . .	1.43	1.76	39	22				
Crude fibre . . .	29.20	32.0	71	74			3.49	
N-free extract . .	48.97	42.31	61	55				
Total carbohydrates	78.17	74.31	64	63				28.1

Calcium, phosphorus and nitrogen balances under aridial feeding.—It has already been stated that the aquatic grasses are normally calcium poor and hence all the animals have shown a deficit calcium balance. The data are shown in the following Table :—

TABLE XIV
Calcium, phosphorus and nitrogen balances

Animal Number	Intake grams	Voided in faeces grams	Voided in urine grams	Total voided grams	Balance grams
<i>Calcium</i>					
1	6.99	8.16	0.10	8.26	-1.27
2	5.96	7.32	0.15	7.48	-1.52
3	5.87	7.70	0.08	7.78	-1.91
<i>Phosphorus</i>					
1	7.61	7.01	0.10	7.11	+0.50
2	6.48	6.17	0.22	6.39	+0.09
3	6.39	5.70	0.09	5.85	+0.54
<i>Nitrogen</i>					
1	50.4	29.6	17.5	47.1	+3.3
2	50.6	24.4	18.7	43.1	+7.5
3	49.9	26.0	19.9	45.9	+4.0

Mineral nutrition of cattle following the feeding of the aquatic grasses.—Summarising the position on the subject, it is to be found that although the aquatic grasses are fairly rich in those nutrients which are considered useful in the calculation of ration for maintenance and productive purposes, the adverse calcium balance seems to be the limiting factor. For the sake of easy reference, the balance data of the 15 individual tests reported in this paper are shown below :—

TABLE XV

Calcium and phosphorus balances under dal grass feeding

Particular	Animal Number	Calcium		Phosphorus	
		Intake grams	Balance grams	Intake grams	Balance grams
Green dal	1	5.94	-2.76	9.42	-1.04
	2	5.29	-1.29	8.38	-0.40
	3	5.43	-1.59	8.60	-0.45
	4	6.70	-2.88	10.61	-2.28
Dal hay	1	4.74	-1.16	6.25	+2.00
	2	4.51	-0.07	5.95	+0.59
	3	4.05	-0.29	5.33	+0.67
	4	6.10	-1.46	8.05	+3.15
Dal silage	1	7.05	-1.33	3.79	-1.02
	2	6.08	-0.48	3.26	-1.00
	3	7.00	+0.07	3.75	-0.03
	4	8.86	-0.62	4.76	-0.30
Uridal hay	1	6.99	-1.27	7.61	+0.47
	2	5.96	-1.52	6.48	+0.09
	3	5.87	-1.91	6.39	+0.54

It is obvious from the above data that only one animal recorded positive calcium balance. In the case of phosphorus, 50 per cent of the animals under the tests showed negative balance due to obvious reasons. Under green *dal* feeding due to unbalance of calcium and phosphorus ratio, the animals failed to record positive balance. Under silage feeding however, the total intake of phosphorus was so low that a positive balance could not be expected. Finally, it may be stated that when the aquatic grasses constitute the major bulk of the ration, the addition of a suitable calcium salt or some other grasses rich in this mineral is desirable.

SUMMARY

The aquatic grasses are fairly rich in protein and phosphorus but poor in calcium. Even at the worst stage, they are leafy, soft and succulent and contain protein above the hay level. As green fodders they are not much relished by cattle. When made into hay by the 'Tripod system', considerable green colour is retained and the stuff is very much relished by cattle.

Metabolism experiments have shown that the crude fibre digestibility of the grasses is high, which indicates that the fibre is not coarser in ligno-cellulose aggregation.

A positive retention of calcium is not assured whether the grasses are fed as green fodder, hay or silage.

It has been observed that the aquatic grasses should preferably be utilised in the form of hay, as green feeding leads to considerable worm trouble.

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INVESTIGATIONS ON FAMINE RATIONS

JAMAN (EUGENIA JAMBOLANA, LAM.) SEED AS A CATTLE FEED

By

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INDIA is one of the food deficit countries. The shortage of concentrates is much more acute than that of roughages. According to a recent estimate [Kehar, 1946] the supply of concentrates falls short of the adult bovine requirement by about 62 per cent. This estimate does not take into account the growth requirements of young stock and production requirements of milch and draught cattle. The magnitude of the shortage appears all the more serious, when the requirements of 48 million sheep, 38 million goats, 4.5 million equines, 1 million camels and 173 million fowls are also taken into consideration.

With a view to meet this shortage of concentrates Kehar and Chanda [1945] have shown that mango seed kernel, which was largely thrown away as a waste, could be satisfactorily used as a partial substitute for grains and oil cakes in cattle rations, thereby making available 70 million lb. of digestible protein and 760 million lb. of starch equivalent. Likewise, entrails, which are not economically utilized at present, were found by Kehar and Chanda [1947] to be a rich source of protein for cattle and they would make available about 47.4 million lb. of excellent protein material. This article presents observations on the use, as cattle feed, of *Jaman* seeds, which are found in enormous quantities and are at present thrown away as a waste. *Jaman* is an evergreen tree met with throughout India and Burma, ascending the hills to about 6,000 feet. It is also known as Blackplum, *Jamanplum*, *Jambul* and Blackberry, etc.

EXPERIMENTAL

The seeds, collected from the waste heaps or from sweepings under the trees, were washed to remove the adhering sand, dried and analyzed for organic and inorganic constituents. The chemical composition of *jaman* seeds, as also of some common grains, on dry basis is given in Table I.

TABLE I

Composition of jaman seed compared to some common grains on dry basis

—	Crude protein	Ether extract	Fibre	Nitrogen free extract	Ash	Calcium (Ca)	Phosphorus (P)
<i>Jaman seed</i>	8.50	1.18	16.90	51.70	21.72	0.41	0.17
Barley	9.48	1.67	5.23	79.09	4.53	0.18	0.37
Maize	11.11	4.30	1.90	80.66	1.94	0.01	0.41
Oats	10.07	6.55	12.71	65.88	4.79	0.11	0.41
Rice	8.33	0.88	0.38	89.13	1.28	0.16	0.21
Wheat	9.65	1.27	2.41	84.70	1.97	0.23	0.41
Corn *	8.60	3.60	2.00	63.00	1.30	0.01	0.25
Rye *	8.30	1.30	0.60	77.30	0.90	0.02	0.29

*American figures

It will be seen that the seed is fairly rich in crude protein and calcium, the latter being twice that of wheat which is the richest in calcium in this list.

Three adult Kunauni bullocks, identical in age, body weight and general condition, were selected for a long term feeding experiment. They were fed on wheat straw and a concentrate mixture composed of rape cake and *jaman* seed in equal proportions. The animals relished the concentrate after a couple of days and consumed the entire quantity offered to them. Two weeks later, the proportion of *jaman* seed in the concentrate mixture was raised to 75 per cent. The ration given to the animals is shown in Table II.

TABLE II

Ration fed to the experimental animals

Animal Number	Body weight	Rape cake	<i>Jaman</i> seed	Wheat straw	Common salt
1	200	$\frac{1}{2}$ lb.	$\frac{3}{4}$ lb.	6 lb.	1 oz.
2	204	$\frac{1}{2}$ lb.	$\frac{3}{4}$ lb.	6 lb.	1 oz.
3	202	$\frac{1}{2}$ lb.	$\frac{3}{4}$ lb.	6 lb.	1 oz.

The feeding observations extended over a period of 30 weeks. After about five weeks of feeding on this ration a metabolism experiment was conducted by the usual procedure to find out the nutritive value of *jaman* seed.

RESULTS AND DISCUSSION

After a few weeks of feeding this ration, it was observed that the adult animals which ordinarily maintained weight on the Institute scheduled ration started gaining in weight and showed an average increase of 32 lb. at the end of 30 weeks.

TABLE III
Live weight record

Date	Weight in lb.		
	Animal No. 1	Animal No. 2	Animal No. 3
August 22, 1946*	200	204	202
August 29, 1946	200	204	206
September 5, 1946	200	208	212
September 12, 1946	204	212	216
September 19, 1946	208	216	218
September 26, 1946	204	220	220
September 28, 1946	208	226	224
October 8, 1946	208	226	226
October 15, 1946	212	224	224
October 22, 1946	212	226	220
October 29, 1946	212	220	222
November 6, 1946	204	222	226
November 13, 1946	208	228	228
November 20, 1946	204	228	224
November 27, 1946	212	226	226
December 4, 1946	216	224	228
December 11, 1946	212	230	230
December 18, 1946	214	228	226
December 25, 1946	218	224	232
January 1, 1947	218	226	228
January 8, 1947	216	228	230

*Date of first feeding

TABLE III--*contd.**Live weight record*

Date	Weight in lb.		
	Animal No. 1	Animal No. 2	Animal No. 3
January 15, 1947	220	228	232
January 22, 1947	218	230	230
January 29, 1947	220	226	234
February 5, 1947	224	232	234
February 12, 1947	224	228	238
February 19, 1947	224	230	236
February 26, 1947	222	232	232
March 5, 1947	228	234	238
March 12, 1947	228	238	236
March 17, 1947	228	238	236
Gain in weight,	28	34	34

The animals also put on a fine bloom and presented a healthy appearance.

The results showing the digestibility coefficient and nitrogen, calcium and phosphorus balance of the whole ration are given in Tables IV and V respectively.

TABLE IV

Digestibility coefficient of the whole ration

Animal No.	Dry matter	Organic matter	Crude protein	Ether extract	Fibre	Nitrogen free extract	Total carbohydrates
1	49.4	56.0	46.0	58.6	59.4	53.0	56.5
2	47.4	55.6	44.3	54.5	59.7	53.3	56.4
3	47.8	55.9	45.4	57.3	59.8	53.6	56.5
Average	48.2	55.8	45.5	56.8	59.6	53.6	56.5

TABLE V

Nitrogen, calcium and phosphorus balances (in gm.)

	Animal No. 1	Animal No. 2	Animal No. 3
<i>Nitrogen intake :</i>			
Jaman seed	4.13	4.13	4.13
Rape cake	6.22	6.22	6.22
Wheat straw	6.44	8.62	7.48
TOTAL	16.79	18.97	17.83
<i>Nitrogen excretion :</i>			
Faeces	8.93	10.57	9.73
Urine	4.20	5.40	4.92
TOTAL	13.13	15.97	14.65
<i>Nitrogen balance</i>	+3.66	+3.00	+3.18
Biological value of the protein	85.94	83.01	84.07
<i>Calcium intake :</i>			
Jaman seed	1.23	1.23	1.23
Rape cake	0.75	0.75	0.75
Wheat straw	5.18	6.43	6.02
TOTAL	7.16	8.41	8.00
<i>Calcium excretion :</i>			
Faeces	5.71	6.75	6.00
Urine	0.38	0.55	0.55
TOTAL	6.09	7.30	6.55
<i>Calcium balance</i>	+1.07	+1.61	+1.45
<i>Phosphorus intake</i>			
Jaman seed	0.53	0.53	0.53
Rape cake	1.23	1.23	1.23
Wheat straw	1.03	1.38	1.20
TOTAL	1.79	2.14	1.96
<i>Phosphorus excretion :</i>			
Faeces	1.73	1.99	1.85
Urine	0.01	0.01	0.01
TOTAL	1.74	2.00	1.86
<i>Phosphorus balance :</i>	+0.05	+0.14	+0.10

It will be observed that replacement of oil-cake by *jaman* seed to the extent of 75 per cent gives a fairly high positive nitrogen balance. The balances for calcium and phosphorus too are positive. The positive retention of important nutrients agrees with the gain in weight and healthy appearance of the experimental animals.

The data on the consumption, excretion and digestibility coefficients of the various constituents in the *jaman* seeds are detailed in the Table VI. The digestibility was determined by the method of elimination and the necessary data for the auxiliary feeds, viz., wheat straw and rape cake, was taken from Sen [1938].

TABLE VI

Digestibility coefficient of jaman seed

	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
	gm.	gm.	gm.	gm.	gm.	gm.
<i>Bullock No. 1</i>						
Consumed from :						
<i>Jaman</i> seed . . .	237	25.8	3.6	51	157	207
Rape cake . . .	94	38.9	9.6	8	37	46
Wheat straw . . .	1375	40.3	15.8	678	641	1319
TOTAL . . .	1706	105	29	737	835	1572
Voided in faeces :	751	55.8	12	299	384	683
Total digested . . .	955	49.2	17	438	451	889
Digested from :						
<i>Jaman</i> seed . . .	103	16.1	2.6	21	88	125
Rape cake . . .	68	33.1	8.7	3	23	25
Wheat straw . . .	784	<i>Nil</i>	5.7	414	340	729
Digestibility coefficient of <i>jaman</i> seed	43.5	62.4	72.2	41.2	56.1	60.4

TABLE VI—*contd.**Digestibility coefficient of jaman seed*

	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
	gm.	gm.	gm.	gm.	gm.	gm.
Consumed from :	<i>Bullock No. 2</i>					
<i>Jaman</i> seed . . .	237	25.8	3.6	51	157	207
Rape cake . . .	94	38.9	9.6	8	37	46
Wheat straw . . .	1835	53.9	21.1	906	855	1761
TOTAL . . .	2166	118.6	34.3	965	1049	2014
Voided in faeces . . .	961	66.1	15.6	389	490	879
Total digested . . .	1205	52.5	18.7	576	559	1135
Digested from :						
<i>Jaman</i> seed . . .	91	19.4	2.4	21	83	124
Rape cake . . .	68	33.1	8.7	3	23	25
Wheat straw . . .	1046	<i>Nil</i>	7.6	552	453	986
Digestibility coefficient of <i>jaman</i> seed	38.4	75.2	66.7	41.2	52.0	60.2
Consumed from :	<i>Bullock No. 3</i>					
<i>Jaman</i> seed . . .	237	25.8	3.6	51	157	207
Rape cake . . .	94	38.0	9.6	8	37	46
Wheat straw . . .	1599	46.8	18.4	788	745	1533
TOTAL . . .	1930	111.5	31.6	847	939	1786
Voided in faeces . . .	852	60.8	13.5	340	436	777
Total digested . . .	1078	50.6	18.1	507	503	1009
Digested from :						
<i>Jaman</i> seed . . .	98	17.5	2.8	23	85	125
Rape cake . . .	68	33.1	8.7	3	23	25
Wheat straw . . .	912	<i>Nil</i>	6.6	481	395	859
Digestibility coefficient of <i>jaman</i> seed	41.4	67.8	77.8	45.1	54.1	60.4

The digestibility coefficients of the important constituents of *jaman* seed, such as crude protein, ether extract and total carbohydrates have been found to be 68.5, 72.2 and 60.3 respectively. The digestible nutrients per 100 lb. of dry matter as compared with those of other common grains is given in Table VII.

TABLE VII

Digestible nutrients of jaman seed as compared with those of other grains and seeds
(in lb. per 100 lb. dry material)

	Digestible protein	Starch equivalent	Total digestible nutrients
Barley	7.4	84.6	86.0
Maize	8.2	93.3	94.3
Oats	7.8	73.4	78.5
Corn*	6.6	..	74.2
Rye*	7.1	..	87.0
Oats*	7.0	..	72.2
<i>Jaman</i> seed	5.82	45.1	45.63

*American figures

It will be seen that the digestible protein obtained from *jaman* seed is fairly comparable to that of other seeds and grains.

These observations give *jaman* seed a fair place in the list of concentrates and it can be satisfactorily used to replace oil cakes to the extent of about 75 per cent. The keeping quality of *jaman* seed seems to be satisfactory as no deterioration was observed after twelve months storage.

It has not been possible to estimate the quantity of seeds available in India. But it has been found that one tree yields about two to four maunds of seeds annually and it is believed that there are millions of *jaman* trees growing in the plains all over India.

SUMMARY

Investigations were made to find out if *jaman* seed, hitherto rejected as a waste, could be utilised as a feed for livestock. The chemical analyses of both the organic and the inorganic constituents showed that the seed was rich in crude protein and calcium; the latter being twice the amount present in wheat. *Jaman* seed was fed to Kumanni bullocks to the extent of 75 per cent of the total concentrate. The animals showed no disinclination in taking to it. They gained on an average about

32 lb. in body weight and developed a fine bloom during the course of experimental period of 30 weeks. All the animals showed positive nitrogen, calcium and phosphorus balances.

The fairly satisfactory digestibility coefficient of the protein, ether extract and total carbohydrates and the biological value of protein give the seed a place in the list of concentrates of proved value.

It has not been possible to assess accurately the quantity of *jaman* seeds available annually, but as one tree yields about two to four maunds or seeds, it is believed that these observations would make available, from a hitherto unutilized source, millions of maunds of protein-rich food for livestock.

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THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF ALKALI TREATED *BHURRA* GRASS (*SACCHARUM MUNJA*)

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ALKALI treatment of straws has been the subject of study in the Animal Nutrition Section for some time past and it has been observed that alkali treatment improves the nutritive value of roughages. Its effect is mainly on the digestibility of fibre. On alkali treatment the lignin binding of the cellulose of the roughage is ruptured, thereby facilitating the action of digestive juices on cellulose during metabolic processes. Sen, Ray and Talapatra [1942] have shown that on alkali treatment the total carbohydrate digestibility of wheat and paddy straws increased to a marked extent.

In the present work alkali treatment of *Saccharum munja*, locally known as *Bhurra* grass has been studied. In India, where shortage of fodder for livestock is acute, improvement of straws which are not generally utilized by cattle has a great economic significance. *Bhurra* grass is a typical example of such a straw. It is a perennial grass with very low palatability. When green it is not very much relished by cattle and when dry it is impossible to feed it as such. It is available in large quantities and is generally used for thatching purposes. In a previous experiment conducted in the section [by Kehar, 1944], it has been shown that animals could not be induced to eat dry *bhurra* grass except with the addition of molasses and its feeding value, under those conditions of feeding, was found to be 3.8 per cent starch equivalent and 27.78 per cent total digestible nutrients with no digestible protein.

Analysis of the *bhurra* grass has shown that it is fairly comparable with wheat and paddy straws in chemical composition (Table 1, next page). The following work was, therefore, undertaken to see whether dry *bhurra* grass could be improved in its palatability and nutritive values by treating it with caustic soda solution. The results have been found to be quite encouraging.

Treatment of Bhurra grass with caustic soda solution

The *bhurra* grass was chopped and soaked in ten times its weight of 1.5 per cent caustic soda solution in a cemented tank of suitable size for 24 hours. It was turned several times during the period of soaking for a uniform treatment of the lot. Thereafter the residual alkaline liquor was drained off to another tank. The treated straw was washed thrice with water and the washings were run into the mother liquor. It was further washed with water to free it of alkali and the washings were drained out. It was then dried in the sun. Fifty per cent more alkali was

TABLE I
Chemical composition of Bhurra grass, Wheat and Paddy straw
(Percentage expressed on dry matter basis)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Organic matter	Crude protein	Ether extract	Crude fibre	N-free extract.	Total carbon-hydrates	Total Ash	Ash soluble in HCl	Calcium as CaO	Phosphorus as PP_2O_5	Magnesium as MgO	Sodium as Na ₂ O	Potassium as K ₂ O	Chlorine
<i>Bhurra grass</i>	83.59	4.05	2.08	30.99	47.47	87.46	6.51	2.51	0.37	0.88	0.28	0.22	1.17	0.057
<i>Wheat straw</i>	80.83	2.69	0.47	30.13	47.24	80.07	19.17	4.88	0.29	0.09	0.13	0.07	1.13	0.009
<i>Paddy straw</i>	81.57	2.64	1.21	34.79	43.05	77.72	18.45	5.63	0.29	0.12	0.20	0.04	2.51	0.170

added to the mother liquor which was made up to the original volume with water to bring it approximately to the original strength and the reinforced alkaline solution was used for treating another lot of *bhaurra* grass. Twenty-four lb. of caustic soda were used in 1600 lb. of water for treating 160 lb. of *bhaurra* grass. Twelve lb. more of caustic soda were used to reinforce the residual liquor which was made up to the original volume for treating another 160 lb. of *bhaurra* grass. In all, 36 lb. of caustic soda were used for treating 320 lb. of *bhaurra* grass and the yield of alkali treated *bhaurra* grass was 256 lb. There was thus a loss of 20 per cent in the air dry material due to alkali treatment. The effect of alkali treatment on the dry matter of the straw is as shown below.

TABLE II

Effect of alkali treatment on the dry matter of the straw

Amount taken		Amount obtained after treatment		Per cent dry matter lost.
Air-dry	Oven-dry	Air-dry	Oven-dry	
gm.	gm.	gm.	gm.	
100	92.67	80	72.39	21.88

The loss in dry matter could be accounted for by the loss sustained by the other constituents except crude fibre. Nitrogen free extract and the total ash are the constituents which contributed most to the loss of dry matter.

By the alkali treatment of *bhaurra* grass its various constituents were affected as shown in Table III. The percentage loss or gain of the different constituents are also shown therein. It might be noticed that the percentage of crude protein, ether extract, N-free extract, phosphorus, potassium and chlorine was lowered and that of crude fibre, calcium, magnesium and sodium was increased. Crude fibre was apparently not very much affected by the alkali solution as is evident from the percentage loss. The loss in N-free extract has, however, been compensated by the rise in fibre so that the total carbohydrate content of the treated grass is higher than that of the untreated grass. The rise in the calcium content, may to a great extent, be accounted for by the deposition of calcium on the grass from tap water which on later examination contained significant amounts of calcium. The increase in sodium content of the treated straw was obviously due to a certain amount of the alkali having been retained in spite of the repeated washings.

Digestibility and nutritive value of treated bhaurra grass

For carrying out a digestibility trial two country bulls and two Kumaon hill bulls were used. Their live weights were 833 lb., 832 lb., 231 lb. and 236 lb. respectively. All the animals were started on a ration containing treated *bhaurra* grass fed *ad libitum*, a weighed quantity of 'sarson' cake and an ounce of common salt

TABLE III
Chemical composition of blutra grass and alkali treated blutra grass
(Percentage expressed on dry matter basis)

	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	N-free extract	Total carbohydrates	Total ash	Ash soluble in HCl	Calcium as CaO	Phosphorus as P ₂ O ₅	Magnesium as MgO	Sodium as Na ₂ O	Potassium as K ₂ O	Chlorine
1. <i>Blutra grass</i>	92.97	83.50	4.05	2.08	39.40	47.47	87.46	0.41	2.51	0.37	0.38	0.28	0.22	1.17	0.67
2. <i>Alkali treated blutra grass</i>	90.49	65.37	2.53	0.37	51.12	40.82	91.35	4.93	2.68	0.72	0.10	0.40	0.93	0.58	0.09
3. <i>Percentage loss (-) or gain (+)</i>	-21.88	-29.43	-50.67	-67.56	-11.14	-32.89	-17.85	-43.00	-16.71	+32.04	-0.00	+1.54	+220.0	-74.97	-50.79

per head per day. The ration was fed for a period of 20 days at the end of which faeces and urine bags were fixed on the animals for collecting their faeces and urine. The same ration was continued for another ten days and during this period 24 hourly collections of faeces and urine were made. The samples collected during the experimental period were subjected to chemical analysis with a view to determining the digestibility coefficient and the balance for nitrogen, calcium and phosphorus. Except during the collection period the animals were weighed every three days and found to maintain their body-weight.

The chemical composition of the feeding stuffs, the food consumption per day, and the digestibility determinations of the treated *bhura* grass are shown in Tables IV, V and VI.

TABLE IV

Percentage composition of the feeding stuffs (on dry basis)

	Treated <i>bhura</i> grass	Sarson cake
Crude protein	2.55	33.53
Ether extract	0.87	12.75
Crude fibre	51.12	10.60
N-free extract	40.83	31.88
Total ash	4.63	11.26
Calcium (CaO)	0.72	1.19
Phosphorus (P ₂ O ₅)	0.19	2.08

TABLE V

Food consumption per day (on dry basis)

Animals	Alkali treated <i>bhura</i> grass	Sarson cake	Total consumption
	gm.	gm.	gm.
Country Bull No. 1	4588	650	5238
Country Bull No. 5	4631	650	5281
Hill Bull No. 61	1656	202	1858
Hill Bull No. 334	1791	202	1993

TABLE VI

Digestibility coefficients of alkali-treated bhurra grass

Consumed from	Country Bull No. 1					
	Organic matter	Crude protein	Ether extract	Crude fibre	N-free extract	Total carbohydrates
	1	2	3	4	5	6
	gm.	gm.	gm.	gm.	gm.	gm.
Alkali treated <i>bhurra</i> grass . . .	4,358.17	124.49	42.78	2,303.60	1,887.30	4,190.90
<i>Sawson</i> cake	575.53	211.67	82.24	69.98	211.64	281.62
TOTAL	4,933.70	336.16	125.02	2,373.58	2,098.94	4,472.52
Voided in faeces	1,469.39	188.97	50.28	335.25	894.89	1,230.14
Total digested	3,464.31	147.19	74.74	2,038.33	1,204.05	3,242.38
Digested from <i>sawson</i> cake . . .	443.81	179.92	76.49	30.79	156.61	187.40
Digested from treated <i>bhurra</i> grass .	3,020.50	NH	NH	2,007.54	1,047.44	3,054.98
Digestibility coefficients of treated <i>bhurra</i> grass	69.51	NH	NH	87.15	55.50	72.90

Country Bull No. 5

Consumed from alkali treated <i>bhurra</i> grass	4,398.45	125.64	43.18	2,324.85	1,994.78	4,229.63
<i>Sawson</i> cake	575.53	211.67	82.24	69.98	211.64	281.62
TOTAL	4,973.98	337.31	125.42	2,394.83	2,206.42	4,511.25
Voided in faeces	1,467.16	179.37	48.54	377.66	861.59	1,236.25
Total digested	3,506.82	157.94	76.88	2,017.17	1,344.83	3,275.00
Digested from <i>sawson</i> cake	443.81	179.92	76.49	30.79	156.61	187.40
Digested from treated <i>bhurra</i> grass .	3,063.01	NH	0.39	1,986.38	1,098.22	3,087.60
Digestibility coefficients of treated <i>bhurra</i> grass	69.64	NH	0.00	85.44	57.66	72.93

TABLE VI--*contd.**Digestibility coefficients of alkali-treated bhurra grass.*

Consumed from	Hill Bull No. 61					
	Organic matter	Crude protein	Ether extract	Crude fibre	N-free extract	Total carbohydrates
	1	2	3	4	5	6
	gm.	gm.	gm.	gm.	gm.	gm.
Consumed from alkali treated <i>bhurra</i> grass	1,585.82	30.61	13.46	861.53	671.22	1,532.75
<i>Sarson</i> cake	179.34	69.52	25.87	21.05	62.00	83.95
TOTAL	1,765.16	109.13	39.33	882.58	733.12	1,616.70
Voided in faeces	547.02	63.22	16.61	163.53	303.66	467.19
Total digested	1,218.14	45.91	22.72	719.05	439.46	1,149.51
Digested from <i>sarson</i> cake	138.96	59.09	24.06	9.26	46.55	55.81
Digested from treated <i>bhurra</i> grass	1,079.18	Nil	Nil	709.79	382.91	1,093.70
Digestibility coefficient of treated <i>bhurra</i> grass	68.05	Nil	Nil	82.39	57.20	71.36
Hill Bull No. 334						
Consumed from alkali treated <i>bhurra</i> grass	1,714.89	42.84	14.56	931.67	725.82	1,657.49
<i>Sarson</i> cake	179.34	69.52	25.87	21.05	62.00	83.95
TOTAL	1,894.23	112.36	40.43	952.7	788.72	1,741.44
Voided in faeces	605.20	72.80	19.34	182.47	330.59	513.06
Total digested	1,289.03	39.56	21.09	770.25	458.13	1,228.38
Digested from <i>sarson</i> cake	138.96	59.09	24.06	9.26	46.55	55.81
Digested from alkali treated <i>bhurra</i> grass	1,150.07	Nil	Nil	760.99	411.58	1,172.57
Digestibility coefficients of treated <i>bhurra</i> grass	67.07	Nil	Nil	81.68	56.71	70.74

The average digestibility coefficients of the organic nutrients of the treated *bhurra* grass are shown in Table VII.

TABLE VII
Digestibility coefficients of treated bhurra grass

1 Animals	2 Organic matter	3 Crude protein	4 Ether extract	5 Crude fibre	6 N-free extract	7 Total carbohydrates
C.B. No. 1	69.31	<i>Nil</i>	<i>Nil</i>	87.15	55.60	72.90
C.B. No. 5	69.64	<i>Nil</i>	0.99	85.44	57.66	72.93
H.B. No. 61	68.05	<i>Nil</i>	<i>Nil</i>	82.39	57.20	71.36
H.B. No. 334	67.07	<i>Nil</i>	<i>Nil</i>	81.68	56.71	70.74
Average	68.52	84.17	56.77	71.98

The digestibility coefficients were determined by a process of elimination for which the digestibility coefficients for *sarson* cake were taken from Sen [1938]. The animals consumed a fair amount of the treated grass, the average consumption per 100 lb. live weight being approximately 1.4 lb. It may be observed, that the crude protein and the ether extract of the treated *bhurra* grass were not digested by the animals, though in the case of animal No. 5 a slight digestibility of ether extract may be noticed. As regards the digestibility coefficients of crude fibre and total carbohydrates they are fairly high, the figures being 84.17 and 71.98 respectively. When the digestibilities of these ingredients are compared between the country bullocks and hill bulls, it appears that the country bullocks digest the crude fibre and the total carbohydrates slightly better than the hill bulls.

The nutritive value of alkali treated *bhurra* grass has been determined by the usual Kellner's method and the results are set out in Table VIII; for the purposes of comparison the nutritive values of alkali treated wheat straw and paddy straw [Sen, Ray and Talapatra 1942] have also been given.

TABLE VIII

Nutritive values of alkali treated bhurra grass

(Per 100 lb. dry matter)

Straws	D.P.	S.E.	T.D.N.
	lb.	lb.	lb.
1. Alkali treated <i>bhurra</i> grass	Nil	32.4	58.8
2. Alkali treated wheat straw	Nil	34.1	59.0
3. Alkali treated paddy straw	Nil	35.9	55.5
4. Untreated <i>bhurra</i> grass	Nil	3.8	27.8

It may be seen from the Table that the nutritive values of the three alkali treated straws are nearly the same, and that of the untreated *bhurra* grass very low. Even after making allowance for the loss of dry matter and other nutrients by alkali treatment the remainder of the grass has still more S. E. and T. D. N. Calculated on the basis of the original untreated straw these values for the remainder come to 23.5 lb. S. E. and 42.6 lb. T. D. N. ; compared to the untreated grass these values are much higher. The importance of alkali treatment, thus gains more significance. It improves the grass as regards its palatability and nutritive value to a remarkable extent.

The nitrogen, calcium and phosphorus balances under treated *bhurra* grass feeding have also been studied and the balances (average per day) of intake and output of these elements have been worked out and the results are presented in Table IX.

TABLE LX

Nitrogen calcium and phosphorus balances

1	2	3 4		5	6	7
Animal	Intake	Excretion		Total excretion	Balance	Balance per 500 lb. body weight
		Faeces	Urine			
		gm.	gm.			
	gm.	gm.	gm.	gm.	gm.	gm.

Nitrogen						
Country Bull No. 1 . . .	53.79	30.23	13.48	43.71	+10.08	+6.05
Country Bull No. 5 . . .	53.97	28.70	13.65	42.35	+11.62	+6.90
Hill Bull No. 61 . . .	17.46	10.11	3.67	13.78	+3.68	+7.97
Hill Bull No. 334 . . .	17.97	11.65	4.12	15.67	+2.30	+4.88

Calcium (CaO)						
Country Bull No. 1 . . .	40.51	30.23	0.71	30.94	+3.57	+2.14
Country Bull No. 5 . . .	40.81	34.32	1.24	35.56	+5.25	+3.16
Hill Bull No. 61 . . .	14.37	12.05	0.64	12.69	+1.68	+3.64
Hill Bull No. 334 . . .	15.35	12.55	0.57	13.12	+2.23	+4.73

Phosphorus (P ₂ O ₅)						
Country Bull No. 1 . . .	27.43	25.61	0.27	25.88	+1.55	+0.93
Country Bull No. 5 . . .	27.50	24.76	0.21	24.97	+2.53	+1.52
Hill Bull No. 61 . . .	9.47	6.82	0.09	6.91	+2.56	+5.54
Hill Bull No. 334 . . .	9.75	7.79	0.10	7.89	+1.86	3.94

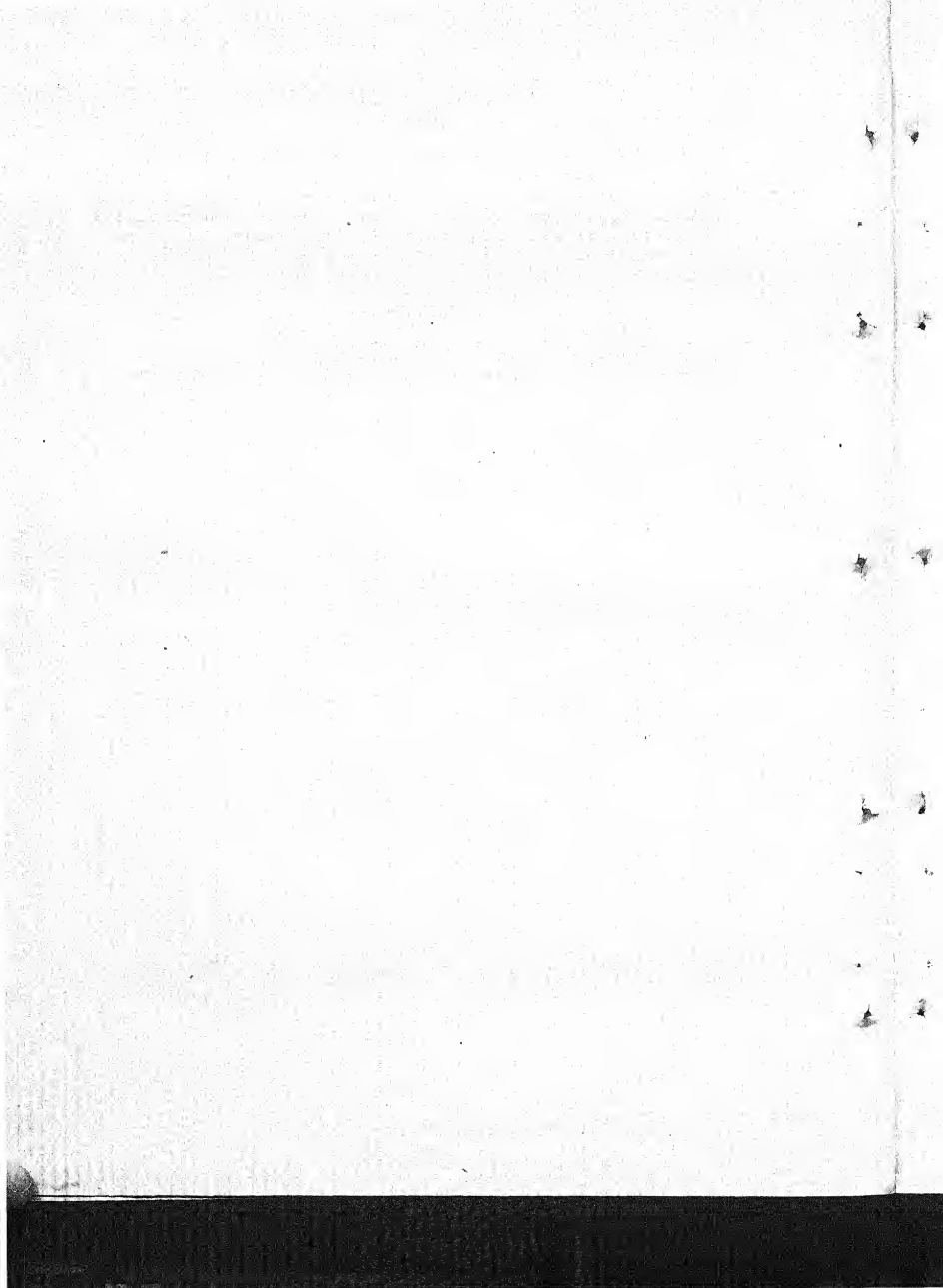
It may be seen from the Table that the balances of nitrogen, calcium and phosphorus are positive in all the cases, the hill bulls showing a greater positive balance in regard to calcium and phosphorus than the country bullocks probably due to a larger ingestion of these nutrients. With regard to nitrogen balance, both these sets of animals do not show any difference. The balances of the three elements under treated *dhurra* grass feeding show that the animals are capable of retaining them in their system.

SUMMARY

Dry *bhurra* grass is not eaten by cattle. Alkali treatment makes it palatable. Cattle consume about 1.4 lb. of alkali treated *bhurra* grass per 100 lb. live weight when it is supplemented with suitable amount of concentrate; and they maintain their live weight and remain in positive nitrogen, calcium and phosphorus balances. The treated grass contains 58.8 per cent of total digestible nutrients and its starch equivalent is 32.4 per cent. In its nutritive value the alkali treated *bhurra* grass compares well with alkali treated wheat and paddy straws.

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INVESTIGATION INTO THE CAUSES OF GENERAL DETERIORATION OF CATTLE IN MALABAR AND SOUTH KANARA (MADRAS PRESIDENCY)

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IT is well known that the cattle on the West Coast of the Madras Presidency are small and stunted in growth which is attributed to mineral and vitamin deficiencies, general malnutrition and parasites, etc. An experiment was conducted to determine the relative importance of each factor, at Pattambi in South Malabar District.

There, in the West Coast, the annual rainfall ranges from 75 to 150 inches, with an average of 100 inches at Pattambi. The soil is poor and laterite in nature and is known to be deficient in lime and phosphates.

There is no distinct breed of cattle in this area, all being non-descript, the average lactation yield of cow being 500 to 700 lb. with a daily average of about 2 lb. and a maximum daily yield of 5 to 6 lb. Stall feeding is not practised as a general rule except in case of a few animals which are fed a little rice gruel and rice straw or in case of milch cattle which get small quantities of concentrates like cotton seed, oil cake and rice bran during their lactation period.

METHODS AND MATERIAL

Forty-eight bull-calves, about one year old and locally bred, were purchased. These were divided into four representative groups of twelve each, keeping in view their size, height, health and vigour so that all the groups were as similar as possible. These groups were named as A, B, C & D and given a different type of feed and treatment. Each group was separately photographed in the beginning of the experiment and thereafter every six months. Their body weights and measurements were kept to see the variation in growth as the result of different feeds and treatments. Group A was the control group and fed rations somewhat similar to that given to the animals of this type normally in this area.

RATION

	Boiled rice (Uncooked)	Fodder (Paddy- straw)	Salt
	lb.	lb.	dr.
1 to 1½ years	½	4	2
1½ years to 2 years 8 months	1	6	2

The quantity of uncooked par-boiled rice shown above was taken and then cooked before administration.

In Group B, the animals were fed on the same ration as Group A, but were dewormed every two months to find the effect of deworming alone on their condition. In Group C, in addition to the food and treatment given to Group B, a mineral mixture and common salt were administered to see the combined effect of deworming and feeding of minerals. Three-quarters of an ounce of salt and one ounce of mineral mixture consisting of sterilised bone meal and fine shellmeal in equal proportions were fed to animals 1 to 1½ years and 1 ounce of salt and 1½ ounces of mineral mixture for animals 1½ years to 2 years 8 months old.

The animals in Group D were fed with a balanced ration shown below and were dewormed every 2 months to see the effects of balanced feeding.

Age of animals.	Oil Cake (groundnut)	Cotton seed	Rice bran	Common salt	Mineral mixture	Paddy- straw	Shark- liver oil
	lb.	lb.	lb.	oz.	oz.	lb.	oz.
1 to 1½ years	½	½	½	½	1	8	½
1½ years to 2 years	¾	¾	¾	1	1	8	1
2 years to 2 years 8 months	1	1	1	1	1	10	1

Groundnut cake was used throughout.

All the groups were allowed exercise for about three hours a day with chances of grazing according to the availability of grass.

The food given to the animals was analysed periodically by the Agricultural Chemist, Agricultural College, Coimbatore, to ensure the same composition throughout the experiment.

The condition of the calves at the commencement of the experiment was very poor. Their coats were very rough and the calves had extensive patches of ring worm.

RESULTS

Verminosis

The greatest trouble that was encountered just before and after the commencement of the experiment was the death among the calves at very short intervals. 11 out of a total of 14 deaths had occurred within a period of two months (from the 18th April 1944 to the 19th June 1944). A large majority of the animals that died both before and after the commencement of the experiment showed clinical symptoms and post-mortem appearances of verminous infection and they were the youngest of the whole lot. However, when the animals attained the age of one year and more and when there was marked improvement in their condition under better feeding, care and management, mortality from verminosis stopped. There were but three deaths for the whole of the remaining period of the experiment, *i.e.*, 18½ months, but the clinical symptoms and post-mortem appearances in these three were quite different from those of the previous 11 deaths.

In all the above 11 cases, there were typical clinical symptoms of verminosis before death. In seven of them, the worms collected at post-mortem from the fourth stomach were identified as *Hecistocirrus digitatus* or *Huemonchus contortus*; thus detection of strongyle eggs in their dung earlier as evidence of verminosis was confirmed at autopsy. It was noteworthy that not a single worm of *Ascaris* species was found and not a single specimen of dung was found to contain eggs of *Ascaris* species even though specimens of dung from each animal were examined regularly throughout the period.

The response shown by the animals suffering from parasitism, particularly with strongylosis, is worthy of attention. It was, however, not possible to save many of these diseased animals from death since in all these, the parasitic infection was so advanced as to cause exhaustion, lowered vitality, anaemia, etc. Every animal that was treated for strongyles with copper sulphate and that died subsequently within a few days of treatment, had no worms at all in the fourth stomach; in a few cases when the period between the treatment and death was short, post-mortem examination revealed either dead or disintegrated worms in the fourth stomach (in one case they were found just at the commencement of the duodenum); whereas in every case that was not treated, numerous worms were discovered in the fourth stomach during autopsy and they were identified as *Hecistocirrus digitatus* or *Huemonchus contortus*. Four of the seven animals that died after the actual commencement of the experiment on 1 May 1944, died early during the experimental period, *i.e.*, from about the fourth to sixth week. Except in one in which there were a good number of *Huemonchus contortus* (this was an untreated case in Group A), no worms were encountered at the time of the post-mortem examination in the other three. The deaths in these cases are considered to be due to the after-effects of strongyle infection coupled with the poor anaemic and exhausted condition of these animals at the beginning of the experiment. That parasitism is particularly harmful in the early age of an animal is again proved by these findings; in addition, it has to be stressed that treatment, to be effective against parasitism, must be undertaken in the early stages of the animals' life, *i.e.*, before it is a year old.

In all the groups except the first which was left untreated as a control, treatment for worms was repeated every three weeks for the first two months and afterwards every two months.

For the first four months, only one per cent solution of copper sulphate in doses of 100 c.c. was given and was found to be effective in the removal of stomach worms. Later on, copper sulphate in combination with nicotine sulphate was given in the following increasing doses, the interval between treatments being two months.

On 21-9-1944

Copper sulphate	gm. 16.5
Nicotine sulphate	c.c. 6.6
Aqua ad.	oz. 33.0
M. Ft. mist.	

Sig/one ounce to each calf

On 23-11-1944

Cupri sulphas	gm. 24.75
Nicotine sulphate	c.c. 6.6
Aqua ad.	oz. 49.5
M. Ft. mist.	

Sig/one and a half ounce to each calf

From 19-1-1945 onwards

Cupri sulphas	gm. 33.0
Nicotine sulphate	c.c. 11.0
Aqua ad.	oz. 99.0
M. Ft. mist.	

Sig/three ounces to each calf

Group A (Control group)

All the animals in this group, without exception, lost or gained weight depending on the availability of green grass in the grazing land.

Three animals in this group died during the course of the experiment, one due to Haemonchosis. In another the clinical symptoms and post-mortem appearances were indicative of parasitic gastritis and the dung taken before death and at post-mortem examination revealed eggs of strongyles but no worms were found. Death in the 3rd case was due to mechanical bronchitis.

From the general appearances of the animals, the post-mortem findings, as well as from the statement of daily weights recorded, it may be observed that the animals in this group were not any more backward than those in Group B, although the latter group was subjected to periodical deworming.

Group B

This group differed from Group A in that the animals in it were dewormed periodically, while in the previous one they were not treated at all.

TABLE I

Statement showing the average live weights with percentages of increase every month of the different groups of animals in the scheme for the improvement of cattle in Malabar and South Kanara (Madras Province) for the period from 1 May 1944 to 31 December 1945

Group	May 1944	June 1944	July 1944	August 1944	September 1944	October 1944	November 1944	December 1944	January 1945	February 1945	March 1945
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
A 1	81.4	81.6	84.6	92.9	105.6	117.2	130.5	138.6	142.7	145.9	145.4
2	..	+0.2	+3.7	+9.8	+13.7	+11.0	+11.3	+6.2	+3.0	+2.2	-0.6
B 1	79.5	79.6	83.0	91.0	104.2	116.0	129.8	137.8	141.3	142.0	141.6
2	..	+0.1	+3.3	+10.7	+13.4	+11.0	+11.9	+6.2	+2.5	+0.18	+0.28
C 1	82.4	83.1	87.0	96.8	108.9	120.7	134.6	142.0	144.2	151.7	151.2
2	..	+1.2	+3.7	+11.8	+13.5	+9.8	+11.4	+6.3	+0.9	+5.2	-0.5
D 1	76.9	80.5	90.4	111.0	128.4	141.6	158.3	172.8	186.2	198.3	209.0
2	..	+12.5	+14.9	+12.6	+14.7	+10.3	+11.8	+9.2	+7.8	+6.5	+5.4

Group	April 1945	May 1945	June 1945	July 1945	August 1945	September 1945	October 1945	November 1945	December 1945	Increase for the whole period	
										Actual increase	Percentage of increase
	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)
A 1	142.4	155.7	157.5	176.2	185.9	192.6	204.7	220.5	231.8	150.4	184.8
2	-2.1	+0.3	+1.2	+11.9	+5.5	+3.6	+6.3	+7.8	+5.1
B 1	138.5	152.1	154.1	173.2	184.6	192.3	202.3	218.6	227.3	147.8	185.9
2	-2.2	+0.8	+1.3	+12.4	+6.6	+4.2	+5.2	+8.1	+4.0
C 1	151.2	166.0	170.9	191.7	203.1	210.9	222.8	240.0	248.4	166.3	202.6
2	..	+9.8	+3.0	+12.2	+5.9	+3.8	+5.6	+7.7	+3.5
D 1	218.4	243.4	250.9	267.3	279.6	289.6	305.6	319.7	330.2	253.3	329.4
2	+4.3	+11.4	+3.1	+6.5	+4.6	+3.6	+5.5	+4.6	+3.3

1. Actual weights (average for the group).

2. Average increase for the group as percentage over previous month's weight.

From the table of summary of weights (Table I) and from the general appearance the progress of this group is more or less similar to Group A, and worm infection does not seem to have materially affected the growth rate or condition of animals. The explanation for this may be that since all the animals had attained the age of about one year at the time the experiment was started, they had perhaps developed a constitution that could not be markedly upset by any worm-infection. Had the animals been as young as three to six months, treatment of the infected ones might have had a pronounced effect as against the untreated ones.

In this group, two animals died showing clinical symptoms and post-mortem appearances of strongyle infection. The deaths can be attributed to the after-effects of parasitism and so treatment could not save these animals. To add to it, there were the factors of lack of pasture and malnutrition to reckon.

Group C

This group differed from Group B in that it was given, in addition, graded doses of a mineral mixture and common salt as shown before. Though the increase in the live weights (*vide* Table I) and shank measurements (*vide* Table II) were a little more in this group than in the previous ones, the results achieved are not very significant.

TABLE II

Statement showing the shank measurements (girth) with percentages of increase every month of the different groups of animals in the scheme for the improvement of cattle in Malabar and South Kanara (Madras Province) for the period from 1 May 1944 to 31 December 1945

Group	May 1944	June 1944	July 1944	August 1944	September 1944	October 1944	November 1944	December 1944	January 1945	February 1945	March 1945
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
A 1				3.36	3.53	3.40	3.68	3.77	3.83	3.90	3.93
2				..	+5.06	+1.98	+2.22	+2.45	+1.60	+1.83	+0.51
B 1				3.30	3.46	3.56	3.64	3.73	3.79	3.85	3.89
2				..	+4.18	+2.89	+2.25	+2.47	+1.61	+1.58	+1.04
C 1	Measurements were taken only from August 1944			3.36	3.54	3.61	3.72	3.82	3.91	3.96	4.03
2				..	+5.37	+2.82	+2.20	+2.60	+2.36	+1.28	+1.53
D 1				3.47	3.68	3.80	3.91	4.08	4.19	4.30	4.40
2				..	+6.05	+3.26	+3.68	+3.55	+2.70	+2.63	+2.33

Group	April 1945	May 1945	June 1945	July 1945	August 1945	September 1945	October 1945	November 1945	December 1945	Increase for the whole period	
	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	Actual increase	Percentage of increase
A 1	3.96	4.00	4.04	4.10	4.18	4.24	4.35	4.39	Measurements were not taken since the animals were disposed off on December 23, 1945	1.03	30.65
2	+1.02	+1.01	+1.00	+1.48	+1.95	+1.44	+2.59	+0.92	
B 1	3.93	3.98	4.00	4.07	4.15	4.22	4.33	4.39		1.09	33.03
2	+1.03	+1.27	+0.50	+1.75	+1.97	+1.60	+2.61	+1.30	
C 1	4.07	4.13	4.18	4.26	4.35	4.45	4.57	4.62		1.56	37.56
2	+1.24	+1.47	+1.21	+1.91	+2.11	+2.39	+2.70	+1.00	
D 1	4.50	4.60	4.67	4.79	4.85	4.93	4.98	5.01		1.54	44.28
2	+2.27	+2.22	+1.52	+1.93	+1.89	+1.65	+1.91	+0.60	

1. Actual measurements (girth) of the shank (average for the group)

2. Average increase for the group as percentage over previous month's measurement

There was one death in this group, during the period, suspected to be due to Babesiosis complicated with pneumonia.

Group D

This group was fed the ration already shown, from the beginning of the experiment until the middle of August 1945, when it was slightly altered on the advice of the Animal Nutrition Committee of the Indian Council of Agricultural Research. This altered diet consisted of a concentrate mixture made up of 7 lb. each of groundnut cake, cotton seed and rice bran and was distributed to each of the 11 animals in the group on the basis of its live weight. Shark liver oil, mineral mixture and salt were continued to be added to the ration in doses originally proposed.

That this group responded to the improved ration given could be seen from the general appearance, the glossiness of the coat, size and vigour of the animals as compared with those of the other groups. The rate of increase of live weight and shank measurements were also greater (*vide* Tables I & II). Maturity as evidenced by sexual desire was noticed earlier in this group than in any other. There was only one death in this group due to strongyle infection at the beginning of the experiment.

ECONOMICS

The actual average cost of feeding one animal in each group is given below :—

	For one day	For the whole period of 602 days
	Rs. a. p.	Rs. a. p.
Group A	0 5 ½	189 11 5
Group B	0 5 ½	189 11 5
Group C	0 5 2	194 13 0
Group D	0 8 0	302 0 0

The straw included in the rations laid down was more than what the animals could consume. The cost of the rations would also be much less in normal times.

The cost of treating one animal at a time for stomach worms with copper sulphate and nicotine sulphate works out to two pises.

Calculation of live weight of animals from the measurements of length and girth

The very large amount of data of measurements and actual weights of more than 40 animals for over 19 months (the weights were noted daily and the measurements once a month) has been utilised to find out the accuracy of the live weights calculated from the formula.

$$W = \frac{L \times G^2}{300}$$

when W is the live weight in pounds, L is the length of the animal

from the point of shoulder to the point of the buttocks in inches, G is the heart-girth of the animal in inches.

The weights calculated from the measurements (length and girth) and actual live weights recorded during a month were averaged. The coefficient of correlation between the actual and calculated weights were worked out monthwise.

The values of coefficients show that the correlation is fairly high, especially so, as the age of the calves advanced. It would, therefore, appear that for all practical purposes the live weight of the animals, when no weighing machine is available, may be calculated by this formula.

SUMMARY

The cattle of the West Coast of the Madras Presidency (Malabar and South Kanara) are small and stunted in growth and cows are poor milkers.

This article deals with a nutritional experiment conducted to study the cause or causes of small and stunted growth of cattle and their poor milk-yield in Pattambi (Malabar), where the rainfall is heavy and the soil is laterite in nature and known to be deficient in calcium and phosphorus.

The experiment was conducted with four groups of one year old locally bred bull calves, A, B, C and D, each group consisting of 12 animals. Group A (the control group) was fed on rice *congee*, paddy straw and a little common salt. Group B received the same ration as above but was dewormed periodically. Group C received the same treatment as Group B but was given, in addition, graded doses of mineral mixture and salt. Group D was fed on a balanced ration consisting of cotton seed, groundnut cake, rice bran, mineral mixture, shark liver oil and salt and was also dewormed periodically. The animals were kept under observation and fed on these rations for a period of 20 months.

The greatest trouble encountered was death among young calves below or nearly one year of age due to strongylosis.

Treatment with copper sulphate alone or in combination with nicotine sulphate was found to be effective.

The animals in Group A were not any more backward than those in Group B although the latter were periodically dewormed showing perhaps, that with advanced age, resistance to the bad effects of helminthic infection also increases.

Rice gruel in quantities given in combination with paddy straw was inadequate for growing calves. Better results were obtained when the same ration was supplemented with green grass.

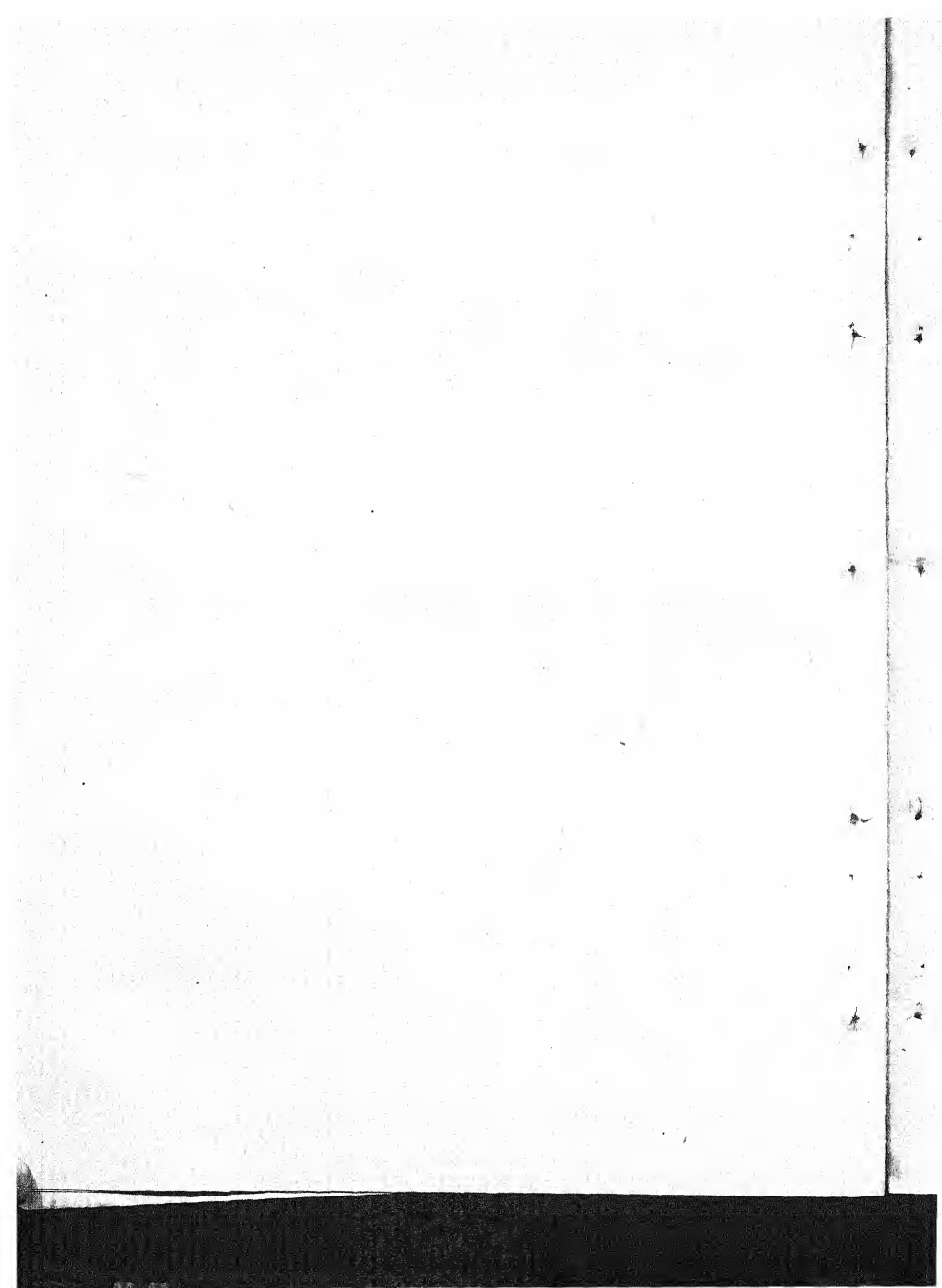
The rate of growth of the animals became less and less as the age advanced.

The feeding of large doses of mineral mixture and salt did not significantly improve the condition of the animals and it is not known whether the inadequate ration or the lack of vitamins A and B or both were responsible for this.

Better results were obtained when an improved balanced ration was given as for Group D.

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TEMPERATURE CORRECTIONS FOR THE LACTOMETER READING OF THE MILK OF INDIAN COWS AND BUFFALOES

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(With one text figure)

IN the routine testing of milk, the determination of the lactometer reading is an essential preliminary test. Seasonal and individual variations in the composition of milk are large, but the herd milk has a comparatively small range in that respect. It is, therefore, not easy to adulterate milk with water to any considerable extent and at the same time keep it within the standard lactometer reading. The simplicity of the test, the cheapness of the equipment required, and the narrow range within which the values fall for genuine milk, render the test quite useful in determining the genuineness of milk.

Ordinarily, to determine the correct specific gravity, the temperature of the milk sample is raised or lowered, as the case may be, to 60°F., before the correct lactometer reading can be taken. This adjustment of temperature is all the more necessary if the sample is below 50°F. or above 70°F. Within this narrow range of 50° to 70°F. fairly accurate values for specific gravity, can be obtained by taking the lactometer reading at the temperature of observation of the sample and then applying the correction factor from the Tables prepared by either Vieth [1885] or Richmond [1899]. These Tables are widely used for finding out the specific gravity of milk at 60°F. from the lactometer reading taken at some other temperature. The total solids may then be calculated from the specific gravity and also the percentage of fat in the milk. The temperature corrections in these Tables, however, range only from 40 to 75°F. and from 40 to 80°F. respectively. The British Standards Institution [1937] has published a correction Table, but even this extends only upto 86.0°F. Hence their use is rather limited in a tropical country like India, where the maximum temperature is seldom below 80°F. for the major part of the year. Wright [1913] extended Vieth's Table with a view to make it applicable to lactometer readings taken between 40°F. and 80°F. Hutchinson [1940] extended Richmond's Table to 90°F. But even these extended Tables do not meet the requirements of countries like India. It is difficult to cool milk samples to 60°F. for specific gravity determinations when the room temperature is 100°F. or above, as it is, in certain seasons. Facilities for cooling milk are seldom available in rural areas. It has, therefore, become necessary to resort to the method of taking the lactometer reading at whatever temperature the milk is received and then applying a correction for the difference in temperatures. The conditions under which this kind of test is required to be carried out demand that the correction Table should be so wide as to cover the range of temperatures usually met with in the tropics.

This paper, therefore, deals with the results of investigations carried out to achieve the above object, as also with a study of the effect of fat content and variation in temperature on the specific gravity of milk. It is a well known fact that a rise in temperature decreases the specific gravity of a milk sample and a fall in temperature increases its specific gravity. Secondly, solids not fat increase the specific gravity, whereas the fat decreases it. Vieth and others have studied the variation of specific gravity mainly with reference to temperature only. Perhaps they have assumed either that fat and solids not fat in milk are almost the same universally or that they vary only within a very narrow range as in the Western countries. In India, however, cow milk shows a wide variation in fat percentage (usually from 3 to 6 per cent), and in buffalo milk a fat percentage as high as 8 per cent is not very uncommon. This makes the study of the problem all the more complicated.

EXPERIMENTAL

Effect of fat and temperature on the correction of lactometer reading

Samples of milk were taken from the dairy herd at the Institute, at 4-30 A.M. and kept under refrigeration at 40°F. till 8-30 A.M. to avoid Recknagel phenomenon. Fat percentage was determined by the Gerber method. The milk samples were then toned to various lower fat percentages with separated milk which usually contained less than 0.1 per cent of fat. Separated cow milk was used for toning cow milk and buffalo milk for buffalo milk.

Generally 200 ml. of the toned milk were taken in a 250 ml. conical flask. After cooling or warming it to the desired temperature it was mixed thoroughly by slow rotation, taking care that no air bubbles were incorporated due to violent shaking. The milk was then poured into a 250 ml. measuring cylinder, the lactometer dipped upto its entire scale length and then left free. When the lactometer came to rest the reading was taken at the top of the meniscus, an addition of 0.2 being made to the reading to make up for the error due to meniscus. Simultaneously with the noting of the lactometer reading the temperature also was noted with a Fahrenheit thermometer.

Of the lactometers available in the laboratory, Gerber's thermolacto-densimeter, graduated from 20 to 40 with a sensitivity of 0.5 and having an attached thermometer ranging from 0° to 40°C., was the only one found to record the same specific gravity as determined by a specific gravity bottle or pycnometer. Another advantage of this lactometer was that its scale divisions were well apart. As, however, the attached thermometer was graduated in degrees C. and as it took considerable time to record the temperature when it was rising or falling, a separate Fahrenheit thermometer was used for noting the temperature of the milk.

Having taken the lactometer reading at 50°F. the temperature of the milk sample was gradually raised to 60°F. by pouring the milk back into the conical flask and heating it on a water bath. Again the lactometer reading was taken as before. These readings were repeated at intervals of 10°F., from 50°F. to 110°F. Whenever the temperature had to be brought down it was done by cooling the sample in ice. After finishing one reading the measuring cylinder and the lactometer were washed and drained completely before another reading was made.

Four dozens of samples each of cow and buffalo milk were studied as described above. Both types of milk are considered together in this paper. The mean of the results of the variations in the specific gravity with fat and temperature obtained, is given below in Table I. All these results were tabulated and the difference in the lactometer reading from that at 60°F. was calculated for every temperature of observation. These differences in lactometer readings always exceeded the correction factors given by Richmond.

TABLE I

Fat per cent	Temperature in degrees Fahrenheit	Difference of lactometer reading from that at 60°F.	Fat per cent	Temperature in degrees Fahrenheit	Difference of lactometer reading from that at 60°F.
0.0	50	—	3.0	100	7.0
	70	1.2		110	9.5
	80	2.5		50	1.3
	90	4.3		70	1.5
	100	6.4		80	3.2
	110	8.4		100	7.2
1.0	50	—	4.0	110	9.5
	70	1.4		50	1.5
	80	2.8		70	1.5
	90	4.5		80	3.4
	100	6.7		90	5.3
	110	9.2		100	7.5
2.0	50	—		110	9.8
	70	1.4		—	—
	80	2.9		—	—
	90	4.7			

From Table I, it will be seen that the higher the fat percentage, the higher the variation of the specific gravity, with increase of temperature. As the milk from the Indian cows and buffaloes has a high and variable fat percentage this factor assumes great importance. It is, therefore, to be expected that Richmond's Correction Table would not give the exact correction factors under conditions such as are prevailing in India. This obviously makes the preparation of a fresh correction table, covering both the temperature ranges and the fat percentage usually met with in India, very necessary. The temperature range for the Table in question was

fixed from 50°F. to 110°F. The fat percentage was fixed at 3 as the minimum, since no genuine milk sample is ordinarily below this figure in India. The higher limit of fat was fixed at 8 as ordinarily the fat percentage does not exceed this.

Preparation of the Correction Table

One hundred and seventy-five samples covering a range of 3.0 to 8.5 per cent fat were studied. These included approximately equal numbers of both cow and buffalo milk samples. The temperature range adopted was 50°F. to 110°F. with readings at intervals of 10°F. The difference in lactometer reading at each temperature interval from the lactometer reading at 60°F. was thus experimentally obtained for each fat percentage.

These results were all grouped according to the fat percentage and the mean of all the values was taken in each case as the average difference of lactometer reading from the lactometer reading at 60°F. at the particular temperature and for that particular fat percentage. These mean differences for each fat percentage at 50°, 70°, 80°, 90°, 100°, and 110°F. were plotted and presented in Fig. 1. This graph was utilized for preparing Table II for correcting the lactometer reading taken at any temperature between 50°F. and 110°F. for milk samples with fat percentage ranging from 3 to 8.

TABLE II

Table for correcting Lactometer reading to 60°F.

Temperature in degrees. Fahrenheit	Percentage of fat observed					
	3	4	5	6	7	8
Corrections to correct L.R. to 60°F.						
50	1.3	1.5	1.6	1.6	1.9	2.0
51	1.2	1.3	1.4	1.4	1.7	1.8
52	1.0	1.2	1.3	1.3	1.5	1.6
53	0.9	1.0	1.1	1.1	1.3	1.4
54	0.8	0.9	1.0	1.0	1.1	1.2
55	0.7	0.8	0.8	0.8	0.9	1.0
56	0.5	0.6	0.7	0.7	0.8	0.8
57	0.4	0.5	0.5	0.5	0.6	0.6
58	0.3	0.3	0.3	0.3	0.4	0.4
59	0.1	0.2	0.2	0.2	0.2	0.2
60						

To be subtracted from
the L.R.

TABLE II—*contd.**Table for correcting Lactometer reading to 60° F.*

Temperature in degrees Fahrenheit	Percentage of fat observed					
	3	4	5	6	7	8
	Corrections to correct L.R. to 60°F.					
61	0.1	0.1	0.2	0.2	0.2	0.2
62	0.3	0.3	0.4	0.4	0.4	0.4
63	0.5	0.5	0.6	0.6	0.6	0.6
64	0.6	0.6	0.8	0.8	0.8	0.8
65	0.7	0.7	1.0	1.0	1.0	1.0
66	0.9	0.9	1.2	1.2	1.2	1.2
67	1.1	1.1	1.4	1.4	1.5	1.5
68	1.2	1.2	1.5	1.5	1.6	1.6
69	1.4	1.4	1.7	1.7	1.8	1.8
70	1.5	1.5	1.9	1.9	2.0	2.0
71	1.7	1.7	2.1	2.1	2.2	2.2
72	1.9	1.9	2.3	2.3	2.4	2.4
73	2.0	2.1	2.5	2.5	2.6	2.6
74	To be added to the L.R.	2.2	2.3	2.7	2.7	2.9
75		2.3	2.5	2.9	2.9	3.1
76		2.5	2.7	3.1	3.1	3.3
77		2.7	2.9	3.3	3.3	3.5
78		2.9	3.1	3.5	3.5	3.7
79		3.0	3.2	3.7	3.7	3.9
80		3.2	3.4	3.9	3.9	4.1
81		3.4	3.6	4.1	4.1	4.4
82		3.6	3.8	4.3	4.3	4.6
83		3.8	4.0	4.5	4.6	4.8
84		4.0	4.2	4.7	4.8	5.0
85		4.2	4.4	4.9	5.0	5.2

TABLE II—*contd.**Table for correcting Lactometer reading to 60° F.*

Temperature in degrees Fahrenheit	Percentage of fat observed					
	3	4	5	6	7	8
Corrections to correct L.R. to 60° F.						
86	4.4	4.6	5.1	5.2	5.3	5.4
87	4.6	4.8	5.2	5.4	5.6	5.7
88	4.8	4.9	5.4	5.6	5.8	5.9
89	4.9	5.1	5.6	5.8	6.0	6.1
90	5.1	5.3	5.8	6.0	6.2	6.3
91	5.3	5.5	6.0	6.2	6.4	6.6
92	5.5	5.7	6.2	6.5	6.7	6.8
93	5.7	6.0	6.4	6.7	6.9	7.1
94	5.9	6.2	6.6	6.9	7.1	7.3
95	6.1	6.4	6.9	7.2	7.4	7.6
96	6.4	6.6	7.1	7.4	7.6	7.8
97	6.6	6.9	7.3	7.7	7.9	8.1
98	6.8	7.1	7.5	7.9	8.3	8.3
99	7.0	7.3	7.7	8.2	8.4	8.6
100	7.2	7.5	7.9	8.4	8.6	8.8
101	7.4	7.7	8.2	8.6	8.8	9.1
102	7.6	7.9	8.4	8.8	9.0	9.3
103	7.8	8.2	8.6	9.0	9.2	9.5
104	8.0	8.4	8.8	9.2	9.5	9.8
105	8.3	8.6	9.0	9.5	9.7	10.0
106	8.5	8.9	9.3	9.7	9.9	10.2
107	8.7	9.1	9.5	9.9	10.1	10.3
108	9.0	9.3	9.7	10.1	10.3	10.7
109	9.2	9.6	10.0	10.4	10.6	11.0
110	9.4	9.8	10.2	10.6	10.8	11.2

To be added to the L.R.

Lactometer readings were also taken with each increase of 5°F. temperature, and the difference of the lactometer reading from that at 60°F. was then plotted against the temperature. The points were found to be on the same curve as with an interval of 10°F. Cow and buffalo milks of the same fat percentage were studied and were found to behave similarly with regard to the change of lactometer reading with a rise in temperature. Further, cow and buffalo milks were mixed in various proportions and the change in lactometer reading in relation to temperature studied. There again the samples were found to behave quite normally with regard to the correction factors. Milk samples having various acidities ranging from 0.11 to 0.20 per cent lactic acid were also studied in a similar way. It was found that acidity had no effect on the lactometer readings and their corrections.

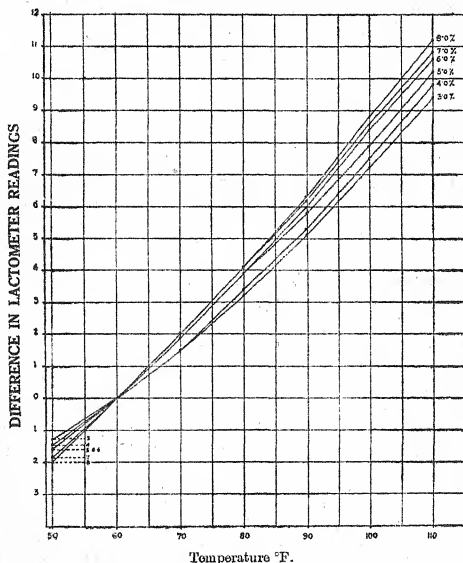


Fig. 1.

VERIFICATION OF THE CORRECTION TABLE

The Correction Table was verified with cow and buffalo milk-samples selected at random. The correct lactometer reading at 60°F., one reading between 50°F. and 60°F., and 2 or 3 readings at temperatures between 60°F. and 110°F. were taken for each sample. The actual difference between the lactometer readings at 60°F. and other temperatures were thus determined and compared with the readings

from the table for the correction of lactometer readings. Over 400 readings were thus experimentally obtained from samples taken at random including cow and buffalo milks and covering the entire range of fat percentages of the table prepared. The summary of the results of this verification showed that 17.0 per cent of the results agreed with corrections found in the prepared table, 22.0 per cent differed by ± 0.1 ; 19.5 per cent differed by ± 0.2 ; 15.0 per cent by ± 0.3 ; 7.0 per cent by ± 0.4 ; 9.0 per cent by ± 0.5 ; and only 10.5 per cent exceeded a difference of ± 0.5 . On the whole, 90.0 per cent of the samples tested agreed within an error of ± 0.5 . Since Richmond's Table extends only up to 80°F. and since he has not mentioned the fat percentage of the milk, the correction factors obtained by Table II can not be strictly compared with Richmond's. However, for milk with 3 per cent fat, the correction factor obtained by our table was found to be much higher than that obtained by Richmond's Table.

Since ordinary lactometers are graduated only in whole numbers indicating only the third place decimal of the specific gravity (0.001), an error of one degree in the lactometer reading may be permitted without much serious inaccuracy in the specific gravity. If such an error is permissible, all the values taken for verifying the new table agree with the Correction Table worked out. Sixty-five readings obtained from the milk of two Ayrshire cows and 150 readings from the milk of half-breed cows were also found to agree with the new table prepared and now recommended as a result of this investigation.

HOW TO USE THE NEW TABLE (ZAL-KRISHNAN TABLE) FOR THE CORRECTION OF LACTOMETER READING

To obtain the correct lactometer reading at 60°F. of any milk sample at any temperature between 50°F. and 110°F., the milk need not be heated or cooled. If the sample is to be examined directly after milking, sufficient time (at least 2 hours) should be allowed, or proper precautions taken to see that the Recknagel phenomenon has ceased. The sample should then be stirred well and the fat percentage of a representative sample determined. The milk should then be poured into a suitable container (preferably a glass container), which should permit free floating of the Gerber's thermolacto-densimeter, without touching either the bottom or the sides. Usually one quarter of a pound of milk will suffice for this purpose, if a cylindrical vessel of suitable diameter is available. The lactometer should then be immersed to the full length of the graduated scale and set free so that it freely moves up and down. When it comes to rest the reading should be noted without any error due to parallax. Simultaneously with the noting of the lactometer reading, the temperature of the sample should also be noted with a good Fahrenheit thermometer. The correction factor should then be read out from the table against the observed temperature and fat percentage, and added or subtracted from the observed reading according to the observed temperature being above or below 60°F. In applying the correction factors the fat percentage may be corrected to the nearest whole number and the correction factor read out from the respective column.

SUMMARY

(a) A new table (Zal-Krishnan) has been prepared for the correction factors of lactometer readings with various temperatures and fat percentages of the milk of Indian cows and buffaloes.

(b) The table provides correction factors for a temperature range of from 50°F. to 110°F.

(c) For the accurate determination of the specific gravity of milk by this method it is essential that the fat percentage should be known.

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FIG. 1. Collection of semen from cock



FIG. 2. Inseminating a hen



FIG. 3. Semen of cock

SEMEN STUDIES AND ARTIFICIAL INSEMINATION IN POULTRY

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(With plate IV and one text figure)

ARTIFICIAL insemination in poultry was first attempted by Ivanoff [1913]. Since his pioneer work various techniques for collection of semen from cocks and for inseminating the hens have been tried by several workers abroad. However, artificial insemination in poultry has not assumed the same importance that it has in large farm animals. The reasons are that most of the Western countries have already reached a very high and uniform standard of excellence in poultry and production costs in poultry are nominal as compared to those in large farm animals. Furthermore, there is no great scarcity of high quality cocks for breeding purposes. Also fertile pedigree eggs can be had easily. The life-cycle in these animals is very short and huge flocks can be developed in about a couple of years.

In India the conditions are different. Poultry husbandry is yet in its infancy. The size of birds is small and their performances poor. Good birds are rare. By the introduction of artificial insemination, a large number of hens can be bred from a few outstanding cocks. Also, it will remove the difficulty of physical incompatibility encountered in mating small sized hens with large sized cocks. To carry out a successful programme of breed improvement by grading and other breeding practices information regarding the characteristics of cock semen is needed.

The present experiment was undertaken to determine the characteristics of semen of *desi* cocks and exotic birds (White Leghorns) reared in India.

MATERIAL

The experimental material consisted of three one year old White Leghorns, an equal number of *desi* birds of the same age, and 13 laying hens. Of the 13 hens six were White Leghorns and the remaining Rhode Island Reds. The birds were obtained from the poultry farm, Indian Veterinary Research Institute, Izatnagar. The hens were housed in a shed 14 ft. 3 in. \times 11 ft. opening on one side into a run 18 ft. 6 in. \times 14 ft. The cocks were kept confined in separate cages 1 ft. 7½ in. \times 1 ft. 4½ in. \times 2 ft 5 in. throughout the experimental period, except at the time of semen collection. Care was taken that each cock received sufficient sunlight. The birds were kept on a balanced ration throughout the experimental period.

METHOD

Collection of semen from cocks and insemination of hens were made according to the method of Burrows and Quinn [1935]. The semen is collected by massaging the soft part of the abdomen below the pelvic bones of a cock as shown in Plate IV, fig. 1 and for inseminating the oviductal opening of the hen is first exposed by

TABLE I
Characteristics of semen produced by Desi and White Leghorn cocks

Cock No. and their breeds	No. of samples studied	Colour and consistency		Volume (in c.c.)			pH			Initial motility		
		Range	Most common	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Most common
114 <i>Desi</i>	25	Thin milky to thick milky	Thick milky	0.14	0.5	0.335 ± 0.018	0.6	7.0	6.8 ± 0.03	++++	++++	+++++
158 <i>Desi</i>	25	Very thin milky to thick milky	Very thin milky	0.12	0.51	0.236 ± 0.019	0.6	7.2	6.8 ± 0.02	++	+++++	++++ to +++++
2320 <i>Desi</i>	25	Thin milky to thick milky	milky	0.11	0.35	0.182 ± 0.011	0.2	7.0	6.6 ± 0.03	++	+++++	+++++
667 White Leghorn	25	Very thin milky to very thick milky	Very thick milky	0.2	0.5	0.304 ± 0.012	5.7	7.4	6.8 ± 0.13	++++	+++++	+++++
676 White Leghorn	25	Thin milky to thick milky	Milky	0.23	0.7	0.43 ± 0.025	6.6	7.0	6.7 ± 0.02	++++	+++++	+++++
664 White Leghorn	25	Very thin milky to very thick milky	Very thick milky	0.13	0.9	0.378 ± 0.0376	6.6	7.6	6.4 ± 0.46	+	+++++	+++++

TABLE I—*contd.**Characteristics of semen produced by desi and White Leghorn cocks*

Cock No. and their breeds	No. of samples studied	Colour and consistency		No. of sperms per c.c. (in millions)			Total No. of sperms (in millions)			Percentage of abnormal spermatozoa		
		Range	Most common	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
114 Desi	25	Thin milky to thick milky	Thick milky	1390	4500	3140 ± 107	115.2	2240	1012 ± 104	2	6	4.08 ± 0.288
158 Desi	25	Very thin milky to thick milky	Very thin milky	152	3920	1635 ± 553	12	840	401 ± 77	2	0	8.84 ± 0.214
2320 Desi	25	Thin milky to thick milky	Milky	1040	5360	3287 ± 240	208	1120.4	917 ± 68	1	6	5.2 ± 0.172
667 White Leghorn	25	Very thin milky to very thick milky	Very thick milky	640	5240	3532 ± 185	160	2400	1191 ± 92	0	6	8.8 ± 0.20
676 White Leghorn	25	Thin milky to thick milky	Milky	1220	5120	3624 ± 190	580	1888	1256 ± 232	0	7	3.72 ± 0.254
664 White Leghorn	25	Very thin milky to very thick milky	Very thin milky	44	5560	2801 ± 547	5.72	3744	1366 ± 190	0	7	3.08 ± 0.31

TABLE II

Significance in semen characteristics of the two breeds

Semen characteristics	Mean of White Leghorn	Mean of Desi breed	Difference of the two means	Standard error of the mean	t. value
Volume in c.c.	0.37	0.26	0.11	0.025	4.40**
pH	6.686	6.833	0.147	0.1634	1.11
Initial motility	4.7	4.57	0.13	0.152	0.85
No. of spermatozoa per c.c. (in millions)	3256	2706	550	211	2.606*
Total number of spermatozoa per collection	1237	773	464	38	12.2**
Percentage of abnormal spermatozoa	3.62	3.72	0.10	0.25	0.4

TABLE III

Fertilizing quality of semen produced by White Leghorn and desi cocks

Cock No. and breed	Mated to hen No. and breed	Total No. of eggs laid	Total No. of fertile eggs	Percentage of fertility	Average percentage of fertility
664 White Leghorn	342 (R. I)	47	42	89.3	
	424 (W. L)				
667 White Leghorn	742 (R. I)	42	38	85.7	88.73
	709 (W. L)				
676 White Leghorn	328 (R. I)	71	62	87.2	
	340 (R. I)				
	422 (W. L)				
114 Desi	381 (W. L)	59	52	88.14	
	341				
158 Desi	603 (W. L)	116	97	83.5	82.21
	607 (R. I)				
	358 (W. L)				
2320 Desi	530 (R. I)	40	30	75.0	

W. L—White Leghorn
R. I—Rhode Island Red

as given by Lambert and Mackenzie [1940]. As regards the percentage of abnormal spermatozoa there was no wide variation between the two breeds. The mean percentage of abnormal spermatozoa did not exceed more than five per cent. Sampson and Warren [1939] also found that in males of proved normal fertility the abnormalities of sperm were up to five per cent.

Burrows and Quinn [1937] obtained 90 per cent fertility in fowls by inseminating one hen per week with 0.1 c.c. of good quality semen. Jeffrey [1941] reported 82 per cent fertility in hens by inseminating once a week with the same amount. It seems, therefore, that percentage of fertility may vary from 82 to 90 per cent in fowls by one insemination a week with 0.1 c.c. of good quality of semen. The percentage of fertility obtained for individual birds as well as for the two breeds were almost the same as reported by the above mentioned workers. The difference in percentages of fertility as found in *desi* and White Leghorn cocks may not be due to the difference in the semen quality of the two breeds.

SUMMARY

Various characteristics of semen of White Leghorns and *desi* birds have been studied.

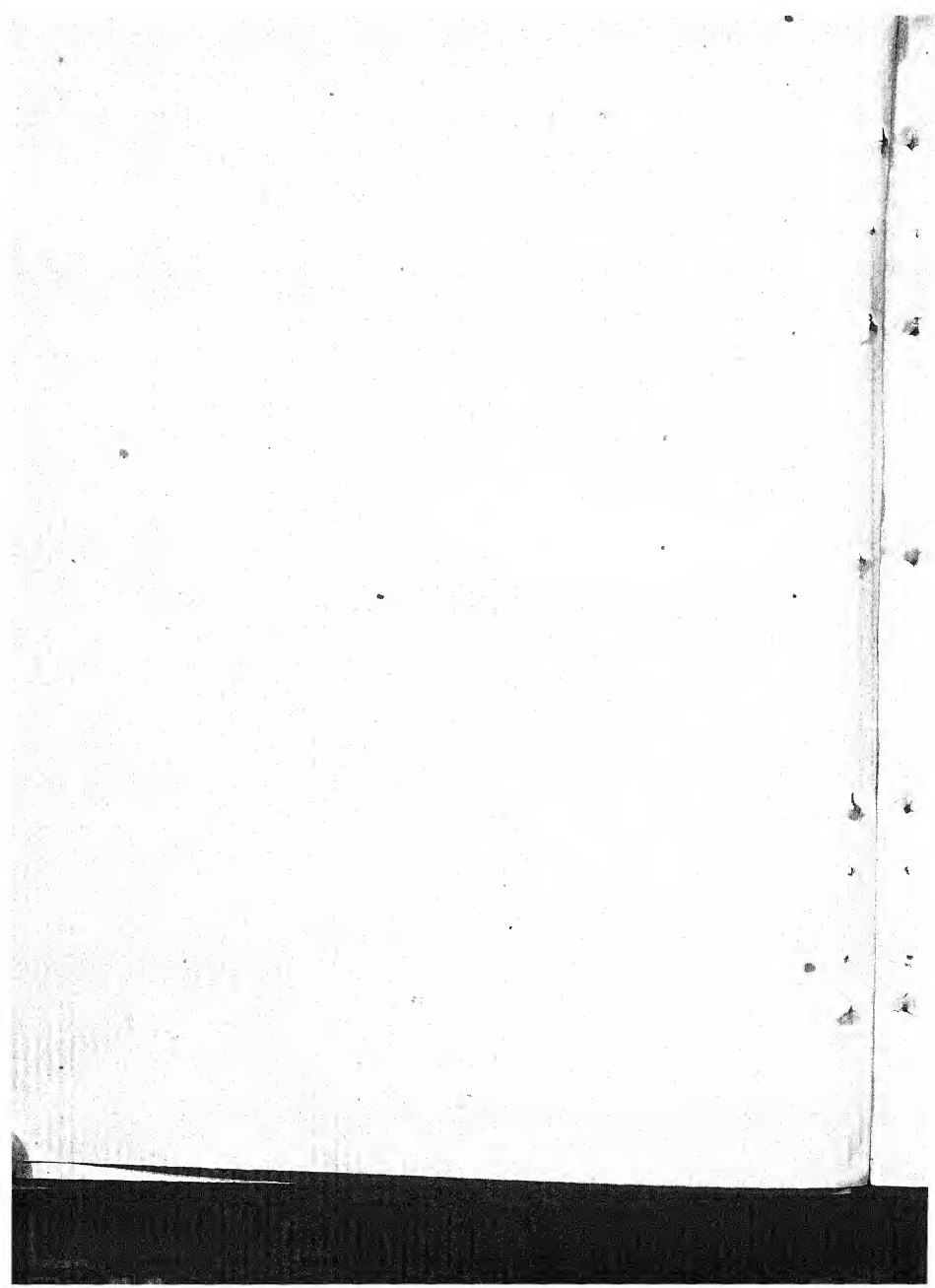
Significant variation was found in volume, total number of spermatozoa and concentration of spermatozoa in the two breeds—White Leghorn being superior to *desi* birds.

No significant variation was found in pH, initial motility and percentage of abnormal spermatozoa.

The percentage of fertile eggs laid by birds inseminated with semen from *desi* cocks was 82.21 against 88.73 in those inseminated with White Leghorn's semen.

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ABSTRACTS

COCCIDIOSIS OF POULTRY. 'SULPHA' DRUGS OFFER NEW HOPE OF CONTROL

Fischel, W.G. (1946). *New Zealand J. Agri.* **73**, (6), 515-517

THE author has found Sulphamezathine to be very useful for the treatment and control of every type of coccidia affecting the poultry. Both forms of administration, *i.e.*, in drinking water and with the feed are equally effective but the former appears to be less wasteful and ensures the intake even if the appetite is impaired by the inflammatory condition of the intestines characteristic of coccidiosis. Saturated solution of the drug is, for example, prepared by dissolving 0.5 gm. (or 8 grains) in hot water by constant stirring. For four-day treatment of hundred chicks of varying ages the proportionate requirement of the drug and water is as follows:

Age 1-2 weeks	10 gm. in 2½ gallons
„ 2-3 „	12 gm. „ 3 „
„ 3-4 „	18 gm. „ 4½ „
„ 4-5 „	24 gm. „ 6 „
„ 5-6 „	34 gm. „ 8½ „

Fresh medicated water should be prepared daily in order to maintain the full strength and should be served in earthenware or glass vessel.

When the drug is given in feed it is recommended to continue the treatment for one week. The drug in the powder form is mixed with the mash at the rate of ½ ounce to every 10 pound of dry mash.

Birds recovering after treatment become immune to infection with the same type of coccidia. (H.S.)

A Virus causing Abnormal Milk in Cattle

Baker, J. A. and Little, R. B. (1946). *Proc. Soc. Exp. Biol. and Med.*, **63** (2), 406-407

THE authors have reported the occurrence of an infection, in dairy cows near Princeton, N. J., characterised by fever and the milk containing flakes composed of polynuclear and mononuclear cells. Most of the cases occurred in the later part of May and in June 1946. Guinea pigs, inoculated intraperitoneally with milk from natural cases, showed thermal reaction and recovered; those killed during the febrile period revealed haemorrhages in the lungs, focal necrosis of the liver and slight enlargement of the mesenteric lymph nodes. Blood from the first and the sixth guinea pig passages was infective to calves and lactating cows. The agent was transferred to rabbits and mice by the intravenous and intraperitoneal routes respectively. The causal factor could also be cultivated on the chorioallantoic

membrane of the developing chick. The authors demonstrated the presence of the agent after ten serial egg passages. No pathological change of the membrane was noticed during the course of such passages.

Attempts to demonstrate the agent microscopically and culturally failed. In view of the above findings it was considered that the infection was due to a virus. (C.S.)

THE AMOUNT OF DDT FOUND IN THE MILK OF COWS FOLLOWING SPRAYING

Howell, D. E., Cave, H. W., Heller, V. G. and Gross, W. G. (1947)

J. Dairy Sci. 30, 717

IN this article the authors have recorded their observations on the amount and toxicity of DDT found in the milk of cows following the recommended and excessive spraying with the drug for the period beginning from July 15 to August 30. Four equal lots, each comprising of two Jerseys and two Holstein dairy cows, were employed in the experiment. The first lot was sprayed daily with 2 quarts of 5 per cent water suspensible DDT spray per animal and the second with a similar quantity of 5 per cent emulsion for the first four days and 1 per cent for the subsequent period of the experiment to prevent tissue damage. The third and the last lots were sprayed individually on the same day every other week, the former with 2 quarts of 0.25 per cent water suspensible powder and the latter with 2 quarts of 0.25 per cent emulsion. In addition to these animals one single cow was sprayed and sampled daily with 2 quarts of 5 per cent water suspensible DDT from August 10 to August 30, to provide more comprehensive data of the elimination of DDT through the milk. It was found that milk samples collected from the experimental cows three and ten days after spraying contained no DDT; but all samples collected on the seventeenth day after spraying was started were positive for qualitative tests. The amount of DDT found in the milk as a result of quantitative tests ranged from slight traces to 33.6 parts per million. The milk of Jerseys generally contained more DDT than those of Holsteins. It was concluded that all animals tested excreted some DDT in the milk and those sprayed with excessive amounts continued to eliminate DDT until the end of their lactation period, 119 and 126 days after the last spraying but none after freshening. Biological tests to determine the toxicity of the milk conducted on mice, mosquito larvae and flies proved to be negative. (T.A.)

A TECHNIQUE FOR THE ISOLATION OF COAGULASE-PRODUCING STAPHYLOCOCCI FROM MILK IN BOVINE MASTITIS

Delia M. Veilla, Faber, M. S. J. E., Jr., Ph. D., and Pelczar, M. J., Jr., Ph. D., College Park, Maryland (1947). *Amer. J. vet. Res.* 8, 275

THE authors have made a comparative study of Difco phenol red, mannitol, salt agar (PRMS), dehydrated (experimental); and Difco Staphylococcus Medium 110 (SM 110), dehydrated (experimental) in isolating pathogenic strains of Staphylococci from milk, taking mainly coagulase-production as the criterion of pathogenicity. Composition of the media is described. Plating was done by spreading

0.1 c.c. of milk, previously shaken, on the medium surface by means of a sterile, bent glass rod. The inoculated plates of PRMS and SM 110 were incubated at 37°C for 36 and 48 hours respectively, and colonies were picked up on the basis of fermentation in the former and pigmentation in the latter. To determine the coagulase activity various methods using fresh, citrated, pooled rabbit blood and plasma were employed. Slide-clumping test was done according to the technique of Cadness-Graves *et al*, and for tube coagulase tests the techniques described by Chapman and Chapman *et al*, were adopted. Observations have also been made regarding haemolytic activity on rabbit blood-agar plates. It has been shown by facts and figures that SM 110 is more efficient than PRMS, although none of the two is perfect. Of all the coagulase tests the slide-clumping test was found to be simple, reliable and quick. Lyophilized blood and plasma proved to be almost as good as fresh blood and plasma on repeating some of the observations with the former. It is further shown that coagulase production does not show complete correlation with either haemolysis or pigment formation on SM 110. All the non-pigmented strains, however, proved to be negative for haemolysis and coagulase production. The procedure recommended for the maximum recovery of coagulase producing *Staphylococci* from milk includes the use of both the media with a final confirmation by slide-clumping test. (G.S.)

THE OPTIMUM STRUCTURE OF BREEDING FLOCKS. I. RATE OF GENETIC IMPROVEMENT UNDER DIFFERENT BREEDING PLANS

Dempster, E.R. and Lerner, I.M., (1947). *Genetics*. 32, 555-66

THE authors have studied the rates of gain in the production index of birds of various age distribution under three different breeding plans. They have stressed that the efficiency of the breeder's operations depends on three factors: (a) the intensity of selection, (b) the accuracy of selection, and (c) the average interval between generations. In the plan designated as III, the first selection is made on two-year old hens, on the basis of the full records of themselves and their sisters. A year later, the second selection from the group thus chosen is made on the basis of their part progeny records. Finally, a third selection is made on the basis of the full records of the progeny at four years of age. In plans I and II, the above procedure is advanced by a year. Under both these plans, the first selection is made on the basis of individual and family records of birds which have been in production for about four months. In plan I, the pullets rejected are not further considered. The second and third selections are similar to that under plan III except that they occur one year earlier. In plan II, the pullets rejected on the basis of their part records are considered at two years of age when they and their sisters have completed records.

As regards males, the first selection is made at one year of age, on the basis of the part records of sisters. At two years of age, the second selection from these males is based on the full records of the sisters as well as the part progeny records.

The authors have shown that greater efficiency is obtained under plans I and II than the schemes under plan III. The use of breeding plans involving a high percentage of pullets, not only leads to more rapid improvement but also result in a

considerable saving in the cost of breeding operations. In addition to the economic savings due to curtailment of trapping operations, certain reproductive economics have also been realized. It is well known that pullets have a higher reproductive rate as regards egg production, fertility and hatchability, and as such a greater number of chicks per breeding bird is obtained, thus permitting higher selection intensity. (S.B.)

NEW INSECTICIDES FOR CHICKEN LICE CONTROL

Telford, H.S., *J. Eco. Entom.* (1945.) (5) 38, 573-576

IN order to meet the urgent need for suitable substitutes for rotenone and pyrethrum, two very efficient and safe lousicides so far then known, which were in short supply during war time, efforts were made to develop effective insecticides for the control of lice on poultry and cattle. Out of 37 compounds tested on 149 chickens infested with lice belonging to the species *Eomeneanthus stramineus* (body louse), *Menopon gallinae* (shaft louse) and *Goniocotes holosericeus* (Hull louse), DDT 0.5 to 4 per cent, derris (with 5 per cent rotenone) 10 per cent and mixtures containing sulphur and the teramethyl thiouram salts exhibited demonstrable residual effect and relatively quick action. Sodium fluoride 33 per cent, kryocide (cryolite) 0 per cent and micronised wettable sulphur were highly effective but slow to act.

The duration of effectiveness of three promising preparations was determined by placing treated hens, freed of lice, in an infested flock. Six birds dusted with 4 per cent DDT had louse infestations approaching their original populations within 30-34 days. Micronised wettable sulphur gave longer protection but 33 per cent sodium fluoride was less lasting. Thanite 5 per cent and lethane B-71, phenothioxine, NH dust and O-nitrodiphenyl each at least were relatively quick acting but demonstrated no residual properties. Unsatisfactory results were given by 15 per cent sodium fluoride, 0.5 per cent nicotine (Black leaf 155), 0.25 and 0.125 per cent DDT, 30 per cent genicide (Xanthone), 5 per cent 2,4-dinitro-O-cyclohexyl phenol, 10 per cent sabadilla, 2 per cent 2,4-dinitro-anisole, a mixture of 2 per cent dinitro-anisole and 2 per cent IN-930, 10 per cent orthophenylphenate and a mixture of 2 per cent di-phenyl and 5 per cent velsicol AR-50.

A proprietary louse powder containing 0.066 per cent pyrethrins, 30 per cent sulphur, 5 per cent naphthalene, 0.45 per cent petroleum hydrocarbons and 64.48 per cent inert ingredients was found to have quick action and good residual effect when undiluted, but unsatisfactory at dilutions of 50 or 25 per cent in pyrax. (P.B.M.)

THE NUMBERS OF DAUGHTERS NECESSARY FOR PROGENY TESTS IN THE FOWL

Muller, C. D. and Hutt, F. B. (1946). *Poultry Science* (25), 246-55

THE report deals with respect to ability to live (viability) and egg production of offspring, in an attempt to determine ways of spotting out superior cocks and hens at early ages and in larger numbers. The breeding season was of 10 weeks' duration and from the first three hatches of the season sufficient number of daughters was usually obtained. When 50 daughters per sire are wanted one should put

15 to 18 females in each breeding pen. Any sires already proven superior by previous progeny tests are recommended to be used continuously through the breeding season, in order to produce the maximum number of superior birds.

In regard to breeding for viability in the fowl the authors endorse the old saying that the sire is more than half the flock, further males are more easily progeny-tested than females. When the mortality in the flock ranged from 37 to 53 per cent in the test period, the records of 86 sires each of which had 50 daughters or more, on analysis, revealed that about 30 daughters were enough for testing the ability of a sire's progeny to live. More daughters were, however, found necessary in the case of the population with lower mortality. Familial differences in resistance to a specific disease (avian leucosis complex) were obvious when it killed 12.6 per cent of the population or more, but not when it killed only 8 per cent. As the numbers of daughters raised from single dams in one season are too small for differentiation of families the authors conclude that tests of dams are less reliable than those of sires. On the other hand the number of daughters necessary for egg laying trials is much less than that required for tests in regard to viability. Six daughters with completed records appeared adequate for tests of dams for ability to transmit egg laying qualities. (S.G.I.)

THE EFFECT OF COBALT ON GROWTH AND CERTAIN BLOOD CONSTITUENTS OF SHEEP

Pope, A. L., Phillips, P. H. and Bohstedt, G. B. (1947). *J. Ani. Sci.* 6. (3)

IN 1944, the problem of low wool and lamb productions arose in Northern Wisconsin. In the beginning it was considered to be a parasite problem complicated by anaemia and a decrease of vitamins A and C in blood plasma. The symptoms were weakness, emaciation, lowering of vitamins A and C and haemoglobin contents. An internal parasitic infection was also noticed. The soil was sandy hence liable to mineral deficiency; it was decided to find out effects of mineral trace elements mixed with salt.

The authors divided the experimental animals (sheep) in two groups which were similar in general condition and body weight. Group I was fed on a ration consisting of iodine, manganese, copper and iron. Group II was given cobalt sulphate in addition to the ration fed to animals of group I. Sheep of group I developed symptoms similar to those noticed in the dying sheep in the country around, while the sheep of group II showed an increase in weight and haemoglobin content.

Later on, cobalt sulphate was added to the ration of group I and it was noticed that the sheep regained their appetite and commenced feeding within three days. After four weeks of feeding they showed an increase in weight, in haemoglobin content and in the concentration of vitamins A and C in the blood plasma. (K.C.)

CONTENT AND DIGESTIBILITY OF MORNING AND EVENING CUTTINGS OF ALFALFA

Adolph, William H., Macdonald, H. A., Avilan Yeh and Lofgreen G. P., August 1947. *J. Ani. Sci.* 3, 6

IN 1944, Curtis reported that alfalfa plots cut in the evening yield 19 per cent more of total dry matter than those cut in the morning. He suggested that there would be a distinct advantage in cutting all the forage crops in the after-noon rather

than in the morning. Further more he felt that the greater part of increase in the dry matter content in the day time was in the easily digestible materials so that there should be a gain in the feeding value much more than that shown by the increase in dry matter. However it was considered desirable to know if feeding tests with animals would show an increase in nutritive value. The present experiment was conducted to compare the digestibility of morning and evening cuttings of alfalfa hay.

For this study a field of alfalfa in the early flowering stage was selected and the plots were statistically treated.

In the above experiment no significant difference in yield was found between the morning and evening cuts, but a significant curing loss was found in the evening cuts.

A metabolism experiment with rabbits showed no significant difference between the digestibility of morning and evening cuts of alfalfa. Oven dried samples of alfalfa showed higher digestibility of carbohydrates, but lower digestibility of protein than the field cured hay. (K.G.)

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ORIGINAL ARTICLES

DURATION OF IMMUNITY IN RINDERPEST

By M. K. SREENIVASAN, G.M.V.C., Veterinary Officer, Indian Veterinary Research Institute, Mukteswar

(Received for publication on 24 May 1948)

NEARLY half a century has passed since the demonstration of the protective value of serum of recovered animals against rinderpest and various methods have been subsequently evolved for the active immunization of cattle against rinderpest. Of all these the serum simultaneous s. s. method was in practice for a very long time and although some simpler and cheaper methods are available, some are in favour of the s. s. method even now. However the goat tissue vaccination has replaced the s. s. method in many parts of India though serum is still used in large quantities either in conjunction with goat blood, goat tissue or bull virus when dealing with highly susceptible breeds of cattle and buffaloes.

Though research work on rinderpest especially on the methods of immunization has been carried out in various parts of the world and millions of animals are immunized every year with one or the other methods in vogue, the question regarding the nature and duration of the immunity produced is still a matter of controversy. Since goat virus came into use the question generally asked by workers in India is whether goat blood virus and the goat tissue virus give the same kind of immunity.

Every one is unanimous in the view that an attack of rinderpest leaves a strong immunity in recovered animals. Thus Hornby and Hall [1925] state that the solidity of rinderpest immunity is one of its most wonderful features. Daubney [1928] observes that formalinised vaccines prepared from spleen pulp confer a high grade active immunity upon inoculated animals. D'Costa and Singh [1938] have reported the duration of immunity in calves immunized by bull virus *cum* serum method as four years (the period tested). Hall [1933] states that notable feature of rinderpest is the strong and lasting immunity which follows an attack and for all practical purposes it can be considered that the immunity is life long. Naik [1938] tested two cattle and two buffaloes that were immunized with goat blood virus after five years with full virus and found them solidly immune. Banerji [1938] tested two cows and one bull which were immunized by serum simultaneous method using bull virus followed by a reinforcing dose of virus and found solid immunity and concludes that the animals immunized by s. s. method followed by a reinforcing dose of virus are liable to develop life long immunity. Khan [1938] and Lall [1945] report that after goat virus vaccination the resulting immunity lasts for 4 and 4½ years respectively (the period tested).

Another factor which requires elucidation is the relationship between the nature of reaction during the primary immunization such as severe, mild, and 'blocked-out' reactions to the resulting immunity. It is well-known that when the primary immunization produces severe or mild reactions the resulting immunity is strong

and lasting but absence of reaction i.e. 'blocked-out' reaction during s.s. method of immunization or vaccination with goat blood or tissue vaccine is due to either an over dose of serum or the virus being so attenuated. In such cases, will the animal develop an immunity and if so what is the nature of immunity? On 'blocked-out' reactions in rinderpest immunizations Carmichael [1928] observes that the dose of serum above a certain minimum is immaterial except from an economic point of view. From Uganda, Egypt, India and elsewhere it has been reported that lasting immunity invariably followed even if no temperature reaction was recorded provided the blood used in the inoculation was virulent. Daubney [1935] states that 'blocked-out' reactors in s.s. method by using serum of high titre again become susceptible to infection as soon as serum immunity has disappeared. D'Costa and Singh [1933] observe on the point whether the duration of immunity bears any relationship to the degree of reaction that arises in the course of immunization, that a durable immunity can be conferred without the appearance of any appreciable symptoms provided the virus is capable of diffusing throughout the body inspite of other factors. Cornell and Evans [1937] state that the so called 'blocked-out' reaction resulting from either vaccine followed by virus or the serum virus s.s. method cannot be relied upon to produce solid and permanent immunity. They suggest that the difference between the blocked-out reactions which result in solid and permanent immunity and those which do not is that the virus had established itself and multiplied in the animals tissue in the former but failed to do so in the latter. Nair, Nilakantan Ayyer and Madhusudan [1939] state that the 'blocked out' reactions do not in any way hamper the production of immunity in buffalo calves.

The plan of the experiments the results of which are tabulated in Table I was laid down about 1931 by the then Director Sir F. Ware and the then Serologist Mr. J. R. Haddow and the author had the opportunity to test the immunity at varying intervals.

The results provide answers to many of the questions generally asked regarding the duration and nature of immunity, when immunized with (i) bull virus and serum (ii) goat blood virus and serum (iii) goat tissue virus alone and (iv) the relation between the nature of reaction at the time of primary immunization and the resulting immunity.

All the animals shown in Table I, especially the hill bulls, were kept isolated in a *kraal* where unimmunized young stock from the cross-bred dairy were kept and there was no outbreak of rinderpest in the *kraal* or near about during the period of observation. There was no possibility of any chance contact with natural disease and the long term immunity could not be due to an unrecognized reinfection at any time after the primary immunization. Many of the bulls were used either for the maintenance of the virus or for testing the serum and on being discontinued were kept isolated for further tests. It is thus seen that when adult animals receive a dose of potent virus with or without serum, irrespective of the reaction produced, they develop a strong immunity which for all practical purposes can be taken as life long.

TABLE I
Details of primary immunization, retest and the duration of immunity against rinderpest

Serial number	Animal number	Details of primary immunization				Details of test				Duration of immunity	Remarks
		Age	Date	Method	Nature of reaction	Age	Date	Method	Result		
1	H. B. 569	3 years	24-9-51	Goat blood virus alone	Severe	10 years	8-12-53	Bull blood	Immune	Over 7 years	
2	H. B. 685	3 years	23-9-51	do.	Rather severe	10 years	8-12-53	do.	do.	Over 7 years	
3	H. B. 492	3 years	23-9-51	do.	Rather severe	10 years	8-12-53	do.	do.	Over 7 years	
4	H. B. 671	3 years	13-1-53	do.	Mild	9 years	1-5-53	do.	do.	Over 6 years	
5	H. B. 933	4 years	8-3-53	do.	Severe	10 years	1-5-53	do.	do.	Over 6 years	
6	H. B. 585	3 years	10-9-54	do.	<i>nil.</i>	8 years	1-5-53	do.	do.	Over 5 years	
7	H. B. 931	3 years	13-1-53	do.	Mild	12 years	12-9-52	do.	do.	Over 9½ years	
8	H. B. 054	4 years	10-3-53	do.	Severe	12 years	12-9-52	do.	do.	Over 9½ years	
9	H. B. 711	5 years	16-6-55	do.	Mild	12 years	12-9-52	do.	do.	Over 7 years	
10	Cow 268	2 months	3-8-55	do.	<i>nil.</i>	11 years	10-7-46	do.	do.	10-11 years	
11	Cow 253	11 months	9-5-55	do.	Mild	13 years	20-6-46	Goat blood*	do.	12 years	*Being in milk and to be on safe side goat serum was used for test for strong bull virus
		3½ months	26-9-53	do.	<i>nil.</i>		10-7-46	Bull blood	do.		
12	Cow 256	1½ years	19-4-55	do.	Mild	12½ years	23-6-46	do.	do.	10-11 years	Mild temperature recorded. Blood not tested.
13	H. B. 105	2½ years	30-6-50	do.	<i>nil.</i>					5 years	
14	H. B. 937	4 years	25-6-54	Goat blood plus serum	Mild	9 years	1-5-53	do.	do.	4 years	
15	H. B. 7	5 years	11-7-54	do.	Mild	10 years	1-5-53	do.	do.	5 years	
		4 years	16-8-54	do.	<i>nil.</i>	9 years	1-5-53	do.	do.		

TABLE I—*contd.*
Details of primary immunization test and the duration of immunity against rinderpest

Serial number	Animal number	Details of primary immunization			Age	Nature of reaction	Details of test				Duration of immunity	Remarks
		Age	Date	Method			Date	Method	Result			
16	H. B. 146	3 years	1-9-34	Goat blood plus serum	13 years	MHR	1-5-39	Bull blood	Immune	5 years		
17	H. B. 11	3 years	11-7-34	do.		nil.	12-9-42	do.	do.	do.	Over 8 years	
18	H. B. 852	3 years	11-7-34	do.		MHR	12-9-42	do.	do.	do.	Over 8 years	
19	H. B. 21	5 years	31-7-34	do.		MHR	12-9-42	do.	do.	do.	Over 8 years	
20	H. B. 1006	3 years	1-9-34	do.	8 years	MHR	12-9-42	do.	do.	Over 8 years		
21	Cow 259	13 months	19-4-35	do.		Severe	13-8-47	do.	do.	do.	Over 11 years	
22	Cow 309	27 months	30-6-36	do.		MHR	20-6-46	Goat blood	do.	About 8 years same as Sl. number 11		
		3½ months	8-10-38	Goat tissue virus alone		MHR	10-7-46	Bull blood	do.			
23	Cow 307	4½ months	8-10-38	do.	10 years	MHR	8-12-49	do.	do.	Over 7 years		
24	H. B. 381	14 months	19-7-39	do.		MHR	1-5-39	do.	do.	do.	Over 4 years	
		6 years	31-1-35	Bull virus plus serum		nil.	1-5-39	do.	do.	do.	Over 4 years	
25	H. B. 459	5 years	31-1-35	do.		nil.	1-5-39	do.	do.	do.	Over 4 years	
26	H. B. 637	6 years	31-1-35	do.	11 years	nil.	12-9-42	do.	do.	Over 7½ years		
27	H. B. 509	7 years	31-1-35	do.		nil.	12-9-42	do.	do.	do.	Over 7½ years	
28	H. B. 525	5 years	31-1-35	do.		nil.	12-9-42	do.	do.	do.	Over 7½ years	
29	H. B. 931	4 years	31-1-35	do.		nil.	12-9-42	do.	do.	do.	Over 7½ years	
30	H. B. 192	5 years	31-1-35	do.	11 years	nil.	12-9-42	do.	do.	Over 7½ years		
31	H. B. 538	5 years	31-1-35	do.		MHR	12-9-42	do.	do.	do.	Over 7½ years	
32	H. B. 264	4 years	31-1-35	do.		nil.	12-9-42	do.	do.	do.	Over 7½ years	
33	H. B. 464	4 years	3-2-35	do.		nil.	15-2-42	do.	do.	do.	Over 7 years	
34	H. B. 543	3 years	3-2-35	do.	MHR	15-2-48	do.	do.	do.	Over 7 years		

From the table it will be seen that of the 34 animals tested, six showed rather severe, 15 mild and 13 blocked-out reactions at the time of primary immunization but all remained solidly immune when tested with a very strong bull virus at varying intervals.*

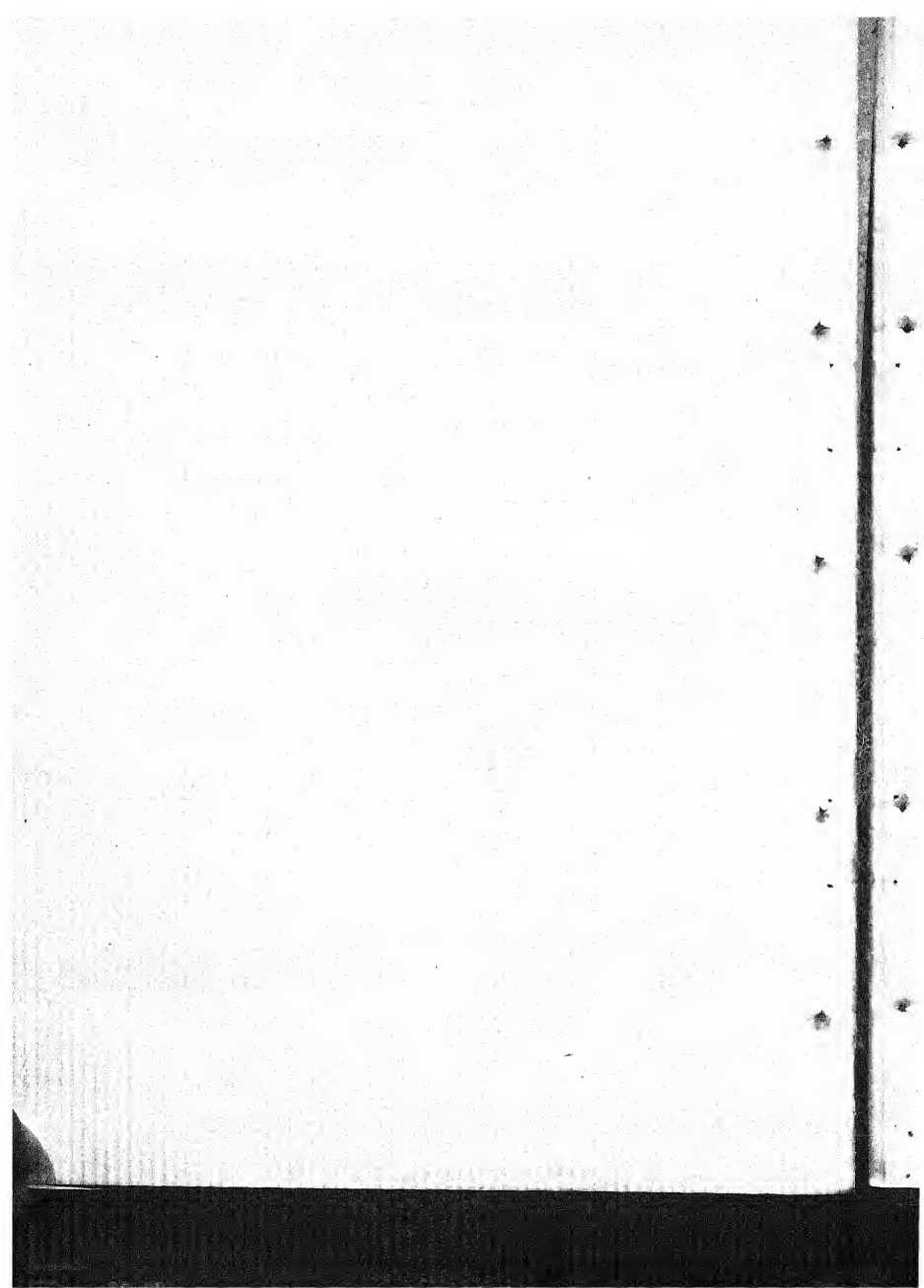
SUMMARY

The literature on the nature of immunity in rinderpest has been reviewed. The table gives the details of primary immunization and the nature of reaction, the results of test and the duration of immunity. It also shows the age at primary immunization and when tested. Of the 34 animals 12 were immunized with goat blood virus alone, nine with goat blood virus and serum, two with goat tissue vaccine alone and 11 with bull virus and serum. Of the 34 animals subjected to test, four were tested after four years, five after five years, two after six years, twelve after seven years, five after eight years, two between 9-10 years, three between 10-11 years and one at twelve years and all remained solidly immune. The virus used for test was a cent per cent potent bull virus. The test results show that the nature of reaction during primary immunization has no relationship to the resulting immunity provided the virus used was potent and that the animals were beyond calfhood when immunized.

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*Since writing the article, the author tested two more animals for immunity the longest period of immunity was found to be in one case 14 years and 8 months and in another 10 years and 11 months.



STUDIES ON PROTEIN METABOLISM

III. AN IMPROVED TECHNIQUE FOR ESTIMATING THE METABOLIC FAECAL NITROGEN OF CATTLE

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(Received for publication on 6 April 1948)

AMONG the methods which have been employed for estimating the biological value of proteins, the Thomas-Mitchell method [Mitchell, 1924] has been most extensively used. But, very few experiments have been conducted on cattle by following this procedure. Further, most of the published experiments on cattle may be open to criticism because, the two pre-requisites viz., endogenous urinary nitrogen and metabolic faecal nitrogen (M.F.N.) of the experimental subjects were not accurately determined. A series of investigations were, therefore, undertaken at this laboratory with a view to evolve a simple procedure for the accurate estimation of these two essentials and for studying the factors which affect their value. The studies concerning endogenous nitrogen have already been published [Kehar, Mukherjee and Sen, 1943]. The present paper deals with the simplified procedure which has been developed for determining M.F.N. of cattle.

Few workers have estimated the M.F.N. of cattle. Titus [1927] computed the M.F.N. of steers by a graphical method, in which the faecal nitrogen (per 100 gm. dry matter intake) was plotted against the nitrogen content of certain rations. These diets were prepared by gradually substituting (upto 60 per cent) of alfalfa by equivalent amounts of 'nitrogen-free' paper-pulp. He showed that under these conditions, the plotted data lie in a straight line and estimated the faecal nitrogen on a 100 per cent paper pulp (i.e., 'nitrogen-free') diet by extrapolation. It is, however, questionable whether extrapolation to this extent (from 60 per cent to 100 per cent paper pulp ration) is permissible. Subsequent workers [Hutchinson and Morris, 1936; Harris *et al.*, 1943] assumed that the faecal nitrogen of cattle on low-nitrogen diets was a fairly correct measure of M.F.N. None of these authors attempted to estimate the error caused by the presence of nitrogen in the experimental ration.

A precise determination of M.F.N., however, requires the feeding of 'nitrogen-free' ration to the experimental animals. Since these are not palatable, workers on non-ruminants have usually included in the experimental ration small quantities of certain proteins, which have been assumed to be almost completely digested at low levels of intake. As a constituent of low-nitrogen rations, these proteins were assumed to exert no appreciable influence on the excretion of metabolic nitrogen in faeces, hence their use was considered permissible. Our present knowledge, however, regarding the protein metabolism of ruminants is so limited that it is not known which feeds will satisfy this condition. To find an answer to this question

it was necessary to run parallel experiments by feeding animals on 'nitrogen-free' and 'low-nitrogen' rations. No successful investigation has so far been made on this line due to the difficulties of preparing and feeding 'nitrogen-free' rations to cattle.

The problem of devising and feeding 'nitrogen-free' diets to cattle has already been investigated by the present authors [cf. Kehar *et al.* 1943]. The following experiments were, therefore, planned to (i) study the faecal nitrogen excretion of cattle on 'nitrogen-free' and 'low-nitrogen' rations, and (ii) devise a method for computing the correct value of M.F.N. from the data obtained by feeding 'low-nitrogen' diets.

EXPERIMENTAL

(a) Rations

The preparations of hot-alkali treated bagasse, cold alkali treated wheat straw dextrinised sago, oil mixture and salt mixture have been dealt with in a previous publication [Kehar *et al.* 1943]. The composition of 'nitrogen-free' ration, low-N ration No. 1 and low-N ration No. 2 is given in Table I.

TABLE I
Composition of rations

Ingredients	N-free ration	Low-N ration No. 1	Low-N ration No. 2
	Parts	Parts	Parts
Sago, dextrinised	52	52	52
Sugar, crystalline (extra white)	6	6	6
Oil mixture	2	2	2
Salt mixture	4	4	4
Bagasse (hot-alkali treated)	36
Wheat straw	36	..
Wheat straw (cold-alkali treated)	36
Nitrogen content	0.02 per cent	0.17 per cent	0.09 per cent

(b) Animals

Two types of adult bullocks, six to eight years of age, were used. These consisted of animals of the Haryana breed [840 to 1210 lb.] and non-descript local bullocks [516 to 630 lb.], the latter being common in the plains of the United Provinces.

(c) *Feeding and management*

The animals were fed once daily. Water and rock salt were available to the animals at all times. The bullocks were weighed on three consecutive days preceding and following a metabolism experiment the average of the six weighings was taken to be the true body weight.

The rations to be used in a particular experiment were prepared in advance, mixed thoroughly and stored. Dry matter and nitrogen contents of the diets were determined occasionally. The rations were offered in weighed quantities daily and the residue was weighed every morning. At the end of every week during the pre-collection period, and at the end of each collection period, composite samples of residue were weighed and the moisture content determined.

The amount of ration offered to each animal was so adjusted that a fairly uniform intake resulted throughout the experimental period. The 'nitrogen-free' diet was not relished by cattle. Hence, hand feeding was sometimes resorted to in order to feed the part of the ration which was left after voluntary consumption. This step was necessary for ensuring uniform food intake, but care was taken not to put any strain on the animals. The subjects soon got accustomed to this method of supplementary feeding.

(d) *Digestibility trials*

According to Mitchell *et al* [1932] there is no necessity for long adjustment and collection periods in digestibility experiments. Since, in our observations faeces markers took three to five days in passing through the alimentary canal of ruminants, adjustment and collection periods of seven to ten days were employed in the present investigation.

The animals were housed in metabolism stalls provided with suitable arrangements for the quantitative collection of faeces free from contamination. Immediately on voiding, the faeces automatically dropped into faeces bags which were attached to the animals. Attendants watched the collection throughout the day and night, and emptied the bags into tared tin containers provided with lids.

Throughout the collection period, the total quantity of faeces excreted by each bullock during the preceding 24-hour period was weighed daily at a particular hour in the morning (usually 10 a.m.). Each sample was then mixed thoroughly in a clean basin and small portions, taken at random from different parts of the heap, were kept in wide-mouthed glass-stoppered bottles. A total of half to one pound of the sampled faeces was sent to the laboratory, where it was thoroughly mixed and aliquot parts weighed out for dry matter and nitrogen estimation.

The aliquots for dry matter, each weighing about 100 gm., were well spread on weighed trays and dried in an electric oven between 105°C. and 110°C. for 24 hours. Dry matter in faeces was estimated daily during the collection period. Separate aliquots of fresh faeces were taken for nitrogen estimation. These aliquots, weighing about 25 gm. per day, were preserved by mixing with 5 c.c. of 25 per cent sulphuric acid each time and kept in a glass stoppered bottle. The contents of the bottle were thoroughly mixed at the end of the experimental period and duplicate samples, weighing ten to fifteen grammes each were finally analyzed for total nitrogen by the Kjeldahl method.

(e) *Laying out of experiments*

In experiment 1, three adult Haryana bullocks were fed the 'nitrogen-free' ration during the adjustment period of ten days and the collection period of one week. In experiment 2, the same animals were fed low-N diet No. 1 during similar adjustment and collection periods. Fresh experiments, viz., experiments 1 (a) and 2 (a) were carried out in order to confirm the results of experiments 1 and 2. In experiment 3, low-N ration No. 2 was used.

Experiments 4, 5 and 6 were similar to experiments 1, 2 and 3 respectively, but were conducted on three local bullocks. Some additional observations were made in connection with experiments 4, 5 and 6; the special procedure adopted for this purpose is given below.

Estimation of undigested feed residue. The following technique, which is an adaptation of the Boas sieve method [cf. Hawk and Bergeim, 1938] for separating the macroscopic constituents of human faeces was adopted for determining the percentage of undigested feed (roughage) residue in the faeces and its contribution to the total faecal nitrogen.

A representative sample of fresh faeces, collected during the previous 24 hours, was employed for this purpose. An aliquot of the faeces, weighing 200 to 250 gm., was placed on a 40 mesh sieve and washed under a slow flowing tap for at least ten minutes after the washings were practically colourless. The washed residue was pressed to remove superfluous water and then quantitatively transferred to a tared tray. The moist feed residue was well-mixed and weighed. One-fourth portions of this, in duplicate, were weighed quickly for total nitrogen estimation and the remaining half was dried in oven. At the same time, 10 to 15 gm., of the untreated fresh faeces was analyzed, in duplicate, for total nitrogen. As usual, the dry matter content of the 24-hour sample of fresh faeces was estimated daily during the collection period.

The particles collected on the sieve may be assumed to represent practically the whole of the undigested particles of roughage, since (i) 90 to 100 per cent of the crude fibre in faeces could be accounted for by crude fibre estimation of the material thus isolated and (ii) microscopical examination of this material, which was carried out on several occasions, showed that it was practically uncontaminated with other faecal matter.

From the data collected according to the above procedure (i) the percentage of undigested feed particles in the faeces in terms of dry matter and (ii) the contribution of the former to the total faecal nitrogen have been calculated. Feed residue analysis was carried out once or twice during each experiment. The average figure of all the animals employed in an experiment was used for calculating the percentage of faecal nitrogen of food origin, i.e., the correction factor applicable to the experiment concerned.

Since this procedure was evolved later, no estimation of feed residue nitrogen was made in course of experiments 1, 2, 2 (a) and 3. Consequently, the figures of 'metabolic' faecal nitrogen for Haryana bullocks which are given in Table V were computed by assuming that the correction factors determined in experiments 4, 5 and 6 on local bullocks could be used for similar experiments on Haryana animals.

without any discrepancy. Further experiments on both types of animals showed that this procedure was justified.

RESULTS AND DISCUSSION

(a) General

The experimental data of Hariana bullocks are recorded in Tables II and III.

TABLE II

Results of experiments on Hariana bullocks

Animal number.	Food D. M. gm.	Faeces D.M. gm.	Digestibility of D. M. per cent	Faecal nitrogen		per 100 gm. faecal D. M. gm.
				Total gm.	per 100 gm. food D. M. gm.	
<i>Experiment 1</i>						
(Ratio : ' N-free ' diet ; 0.02 per cent N)						
Ha 10	5060	1276	74.8	13.2	0.26	1.03
Ha 11	3845	1473	61.7	13.8	0.36	0.94
Ha 13	3800	1400	64.0	12.3	0.32	0.88
<i>Average</i>	66.8	..	0.31	0.95
<i>Experiment 2</i>						
(Ratio : Low-N diet No. 1 ; 0.17 per cent N)						
Ha 10	3735	1860	50.2	17.9	0.48	0.96
Ha 11	4050	2145	47.0	18.9	0.47	0.88
Ha 13	3728	1823	51.1	16.8	0.45	0.92
<i>Average</i>	49.4	..	0.47	0.92

TABLE III

Results of further experiments on Hariana bullocks

Animal number	Food D. M. 'gm.	Faeces D. M. gm.	Digestibility of D. M. per cent	Faecal nitrogen		per 100 gm. faecal D. M. gm.
				Total gm.	per 100 gm. food D. M. gm.	
<i>Experiment 1 (a)</i> (Ration : 'N-free' diet ; 0.02 per cent N)						
Ha 11	3240	935	71.1	8.5	0.26	0.91
Ha 12	3560	1508	57.7	14.8	0.42	0.98
Ha 13	3870	885	77.2	7.6	0.20	0.86
<i>Average</i>	68.7	..	0.29	0.92
<i>Experiment 2 (a)</i> (Ration : Low-N ration No. 1 ; 0.17 per cent N)						
Ha 11	5082	2047	59.7	20.2	0.40	0.99
Ha 12	4864	2224	54.3	20.0	0.41	0.90
Ha 13	4754	2005	57.8	18.5	0.39	0.92
<i>Average</i>	57.3	..	0.40	0.94
<i>Experiment 3</i> (Ration : Low-N ration No. 2 ; 0.09 per cent)						
Ha 10	4091	1335	67.4	13.7	0.33	1.03
Ha 11	5465	2020	63.0	18.1	0.33	0.90
Ha 13	4128	1718	58.4	15.2	0.37	0.89
<i>Average</i>	62.9	..	0.34	0.94

The results obtained in similar experiments on local bullocks are presented in Table IV.

TABLE IV

Results of experiments on local bullocks

Animal number.	Food D. M. gm.	Faeces D.M. gm.	Digestibility of D. M. per cent	Faecal nitrogen		
				Total gm.	Per 100 gm. food D. M. gm.	Per 100 gm. faecal D. M. gm.
<i>Experiment 4</i>						
(Ration : 'N-free' diet ; 0.02 per cent N)						
L 1	2056	1044	64.7	9.4	0.32	0.90
L 5	3005	1056	64.9	9.3	0.31	0.88
L 6	2887	896	69.0	8.4	0.37	0.92
<i>Average</i>	66.2	..	0.33	0.90
<i>Experiment 5</i>						
(Ration : Low-N diet No. 1 ; 0.17 per cent N)						
L 1	2300	1082	53.0	12.0	0.52	1.11
L 5	2390	1165	51.2	11.7	0.49	1.00
L 6	1610	850	47.2	10.1	0.48	1.19
<i>Average</i>	50.5	..	0.50	1.10
<i>Experiment 6</i>						
(Ration : Low-N diet No. 2 ; 0.09 per cent N)						
L 1	2752	983	64.3	10.7	0.39	1.09
L 5	3420	1368	60.0	13.0	0.38	0.95
L 6	2434	1202	50.6	11.2	0.46	0.95
<i>Average</i>	58.3	..	0.41	1.00

In Tables V and VI, the data on both types of animals are summarized and the results of statistical analysis are included.

TABLE V

The relative individual variation of the 'metabolic' faecal nitrogen and other data

Ration	No. of observations	Food D. M./100 lb. body wt. lb.		Faecal D.M./lb. body wt. gm.		'Metabolic' per 100 gm. food D. M. gm.		faecal nitrogen** per 100 gm. faecal D. M. gm.	
		Mean	C. V.*	Mean	C. V.	Mean	C. V.	Mean	C. V.
Hariana bullocks									
'N-free'	6	0.92	8.5	1.36	26.8	0.30	26.2	0.93	6.8
N-Low (1)	6	1.00	20.7	2.09	15.8	0.38	9.0	0.82	4.6
N-low (2)	3	1.04	27.9	1.77	32.9	0.31	7.4	0.87	8.3
Local bullocks									
'N-free'	3	1.17	4.4	1.80	3.6	0.33	9.7	0.90	2.2
N-low (1)	3	0.80	15.1	1.80	10.2	0.44	4.8	0.97	8.8
N-low (2)	3	1.08	9.9	2.04	4.3	0.38	10.0	0.91	8.2

* C. V. is the abbreviation for coefficient of variation.

** Metabolic faecal nitrogen figures have been computed by using appropriate correction factor viz., nil for N-free ration, 12 per cent for N-low ration No. 1 and 8 per cent for N-low ration No. 2.

TABLE VI

Statistical comparison of the observations made with N-free and low-N rations

Observations compared	Mean values		S. D. of difference	't' values			D. F.	Remarks
	M1	M2		calc.	Tabular			
					5 per cent	1 per cent		
<i>Hariana bullocks</i>								
(N-free vs. low-N No. 1)								
Food D. M. (lb./ 100 lb. body wt.	0.92	1.00	0.157	0.83	2.23	3.17	10	Not significant
M. F. N.** (gm./ 100 gm. food D. M.	0.30	0.38	0.061	2.18	2.23	3.17	10	do.
Faecal D. M. (gm./ lb. body wt.	1.36	2.00	0.348	3.65	2.23	3.17	10	Highly significant
M. F. N.** (gm./ 100 gm. faecal D. M.	0.93	0.82	0.052	3.85	2.23	3.17	10	do.

Hariana bullocks

(N-free vs. low-N No. 2)

Food D. M. (lb./100 lb. body wt.)	0.92	1.04	0.169	0.97	2.36	3.50	7	Not significant
M. F. N.** (gm./100 gm. food D. M.)	0.30	0.31	0.068	0.21	2.36	3.50	7	do.
Faecal D. M. (gm./lb. body wt.)	1.36	1.77	0.439	1.33	2.36	3.50	7	do.
M. F. N.** (gm./100 gm. faecal D. M.)	0.93	0.87	0.066	1.43	2.36	3.50	7	do.

** Vide foot note of Table V.

TABLE VI—*contd.**Statistical comparison of the observations made with N-free and low-N rations*

Observations compared	Mean values		S. D. of difference	't' values			D. F.	Remarks
	M1	M2		calc.	Tabular			
					5 per cent	1 per cent		
<i>Local bullocks</i>								
(N-free vs. low-N No. 1)								
Food D. M. (lb./100 lb. body wt.	1.17	0.80	0.096	4.74	2.78	4.60	4	Highly significant
M. F. N.** (gm./100 gm. food D. M.	0.33	0.14	0.027	0.18	2.78	4.60	4	Not significant
Faecal D. M. (gm./lb. body wt.	1.80	1.80	0.138	0.01	2.78	4.60	4	do.
M. F. N.** (gm./100 gm. faecal D. M.	0.90	0.97	0.062	1.38	2.78	4.60	4	do.

<i>Local bullocks</i>								
(N-free vs. low-N No. 2)								
Food D. M. (lb./100 lb. body wt.	1.17	1.08	0.084	1.31	2.78	4.60	4	Not significant
M. F. N.** (gm./100 gm. food D. M.	0.33	0.38	0.025	1.54	2.78	4.60	4	do.
Faecal D. M. (gm./lb. body wt.	1.80	2.01	0.200	1.48	2.78	4.60	4	do.
M. F. N.** (gm./100 gm. faecal D. M.	0.90	0.91	0.035	0.29	2.78	4.60	4	do.

(b) Fallacy of expressing M. F. N.

On food dry matter basis. In experiment 1, the average faecal nitrogen output of the animals was 0.31 gm. per 100 gm. food dry matter consumed. Since the ration fed during the experiment was free from nitrogen, the whole of

the faecal nitrogen is of body origin. The above figure, therefore, represents the M. F. N. A comparison of this figure with the average nitrogen content of faeces in experiment 2 viz., 0.47 gm. per 100 gm. of dry food consumption (Table II) would suggest that the higher value on low-N diet is mainly due to the incomplete digestibility of wheat straw protein. The present day conception of the subject warrants the above interpretation, but it does not appear to be correct in view of the following facts.

The 'N-free' ration differs from the low-N diets not only in nitrogen content but also in the digestibility of dry matter (Tables II, III and IV). By subtracting the digestibility coefficients from 100 we get, let us say, 'coefficients of indigestibility'. It is interesting to note that these latter figures (e.g., 33 per cent and 51 per cent in Table II) run more or less parallel to the corresponding faecal nitrogen data when expressed per 100 gm. food intake (e.g., 0.31 and 0.47 gm.). It suggests that the quantity of indigestible constituents in the ration, and hence the amount of faecal dry matter, is an important factor influencing the nitrogen output in the faeces. For this reason, the faecal nitrogen per 100 gm. of faecal dry matter has also been shown in Table II. On examining these figures alone, no marked difference between the faecal nitrogen data in experiments 1 and 2 is noticed. This throws great doubts regarding the validity of the common method of expressing the M. F. N. of cattle viz., in terms of dry matter consumed. The data given under experiments 1 (a), 2 (a) and 3 (Table III) support the above contention.

(e) *Influence of wheat straw protein on faecal nitrogen*

The most significant point that seems to emerge from experiments 1, 2, 1 (a) and 3 is that the nitrogen content of low-protein rations No. 1 and 2, containing untreated and treated wheat straw respectively, is highly digestible in the case of cattle. The approximate agreement between the faecal nitrogen figures (per 100 gm. of dry faeces) on N-free ration and the two low-N rations lead to this brief (vide Tables II and III). In order to test the validity of this suggestion the experiments on local bullocks (viz; experiments 4, 5 and 6) were so planned that a more clear-cut interpretation of the observations was possible.

Since faecal dry matter excretion rather than food dry matter intake, appeared to be the predominant factor determining the M. F. N. of cattle, it was decided to control the former to a uniform level in experiments 4, 5 and 6. This was attempted by adjusting the food intake of the animals on the basis of the figures given in Tables II and III for the digestibility of the dry matter of the three rations. In these experiments some additional observations were made for estimating the feed residue nitrogen in the faeces according to the method already described. On testing the faeces of the local bullocks fed 'N-free' ration in experiment 4, it was found that feed residue contained only traces of nitrogen (viz., 0.02 per cent N on dry basis) and comprised of less than 1 per cent of the total faecal nitrogen. On the other hand, in experiments 5 and 6, when low-N rations No. 1 and 2 were consumed, the faecal residue contributed 12 per cent and 8 per cent respectively of the faecal nitrogen. Since the non-roughage components of all three rations were common, the undigested remains of the roughage appear to be the practically the only source of residue nitrogen. The above figures, therefore,

indicate that the faecal nitrogen data on the two low-N rations were in excess of the metabolic nitrogen by 12 per cent and 8 per cent only. The low value for the correction factors indicates that the true digestibility of untreated and alkali-treated wheat straw protein is fairly high. It further indicates that low-N rations containing wheat straw, and possibly certain other low-protein roughages, can conveniently be used in place of a N-free ration for the determination of the metabolic faecal nitrogen of cattle by the use of appropriate correction factors depending on the quantity of nitrogen present in the feed residue.

In this connection, it is worthwhile referring to the observations of Hutchinson and Morris [1936] on the effect of roughage on M. F. N. In one of their experiments, they fed three goats on a fibre-free and N-free ration (the feeding was necessarily carried out forcibly). The animals were then offered the same diet plus paper-pulp, when a higher excretion of faecal nitrogen was observed. This finding led the authors to conclude that fibre was responsible for the observed increase in faecal nitrogen. From the results of a subsequent experiment of this type the authors calculated that the increase in faecal nitrogen per 100 gm. of fibre were 1.77, 0.59 and 0.97 gm. (average 1.11 gm.) for three animals; thus the variation between the figures was fairly wide. In a later experiment, goats were fed two rations, one high in straw and the other low in straw. It was found that the difference in faecal nitrogen of animals on the two diets was similar to that which can be expected on the basis of the difference in the fibre content of the rations and the average figure for the increase in faecal nitrogen per 100 gm. of dietary fibre (viz., 1.11 gm.). Hence, the authors concluded that the true digestibility of the nitrogen of straw was almost 100 per cent. Since their interpretation was based on the average figures for the increase in faecal nitrogen per 100 gm. fibre, which was the mean of a small number of widely different values, their conclusion regarding the complete digestibility of straw was not very convincing.

(d) *Statistical analysis of the data*

Table V shows the coefficients of variation of food dry matter, faecal dry matter and the computed figures of metabolic faecal nitrogen (expressed both on food dry matter basis and faecal dry matter basis). It is evident from this table that the variation between individual observations in a given experiment is fairly low for the M. F. N. figures calculated in the new manner viz., on faecal dry matter basis. It indicates that this method of expressing the M. F. N. gives consistent results in the case of cattle. It has been pointed out earlier that the figures expressed in the customary way vary inversely with the coefficient of digestibility of the dry matter of the experimental ration. Strictly speaking, there is little justification, therefore, to refer to these figures as M. F. N.

Table VI indicates that when the observations on one or the other type of cattle are compared according to the nitrogen content of the diet the difference between the M. F. N. figures (on faecal dry matter basis) was significant in only one pair out of four; the difference between the level of faecal dry matter excretion was likewise significant in this pair only. All the statistical results are in agreement with the conclusion, arrived at earlier, on the basis of feed residue analysis, that the M. F. N. figures computed from the data obtained by feeding low-N rations 1 and 2

truly represent the M. F. N. as determined by feeding N-free diet. Since the preparation and feeding of low-N rations are comparatively easier, their use is a very welcome modification.

SUMMARY AND CONCLUSIONS

Two types of cattle were fed a 'nitrogen-free' (0.02 per cent N) and two nitrogen-poor (0.17 per cent and 0.09 per cent N) rations with a view to study the possibility of using low-protein diets in the determination of the metabolic faecal nitrogen. The data suggest that the metabolic nitrogen of cattle should be expressed on faecal dry matter basis, rather than in terms of food dry matter.

The results further indicated that wheat straw protein, which constituted the nitrogenous part of the low-nitrogen rations used, was highly though not completely digested by cattle. A technique was, therefore, developed to measure accurately the error caused by the inclusion of wheat straw in the experimental diet on the value of metabolic faecal nitrogen.

The improved method of metabolic nitrogen determination, thus developed, is recommended, since (a) it is accurate, and (b) the preparation and feeding of low-nitrogen rations is comparatively simpler.

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STUDIES ON PROTEIN METABOLISM

IV.—INFLUENCE OF CERTAIN FACTORS ON THE METABOLIC FAECAL NITROGEN OF CATTLE AND RATS

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[N the preceding paper of this series [Mukherjee and Kehar, 1948], an improved technique for determining the metabolic faecal nitrogen (M. F. N.) of cattle was presented. The present communication is concerned with factors affecting the M. F. N.

PART I

Effect of dry matter intake on M. F. N. of cattle

Mitchell [1924] found that the M. F. N. of rats was fairly proportional to the total dry matter consumption, when rations free of crude fibre were fed. Chick *et al* [1935] and Schneider [1934, 1935] confirmed these findings. Mitchell (*loc cit*) and later Schneider (*loc cit*) observed that, in the case of rats, the value of M. F. N. (expressed in terms of food intake) gradually decreased as the level of dry matter consumption increased. Schneider (*loc cit*) established the reason for the high values of M. F. N. as very low intakes. No information on the effect of this factor on M. F. N. of cattle is available, although Hutchinson and Morris [1936] obtained some evidence regarding the increase of its value at lower levels of food intake in the case of goats and sheep. In view of the lack of knowledge on the subject, the following experiments were conducted.

EXPERIMENTAL

In experiments 1, 2 and 3, three adult Kumaun bullocks were fed a wheat straw ration at three levels of intake. Calcium phosphate was included in the ration to supply the major mineral elements; salt lick was available as usual. The technique adopted in these experiments was similar to that already described [Mukherjee and Kehar, 1946].

RESULTS AND DISCUSSION

Table I shows the results of the experiments mentioned above. The data indicate that the value of M. F. N. tends to decrease at higher levels of food intake; this is true of the figures expressed according to the traditional method (*i.e.*, in terms of food dry matter intake) as well as those calculated according to the present authors' method (*i.e.*, on faecal dry matter basis). Since a ration of constant composition was fed at the different levels, the digestibility of dry matter

was practically constant, and this factor had negligible influence on the results. Hence, in this particular case, the variation in the M. F. N. values stated in terms of faecal dry matter output was only slightly less than the figures expressed per 100 gm. food consumed. These results are expected, because a constant ration was fed in the three experiments. However, the generalized statement that M. F. N. is more accurately expressed on faecal dry matter basis is in no way affected by these results. In fact, this general rule was found to apply to all types of rations investigated by us.

TABLE I
Influence of the level of food consumption on the M. F. N. of cattle
(Data expressed on daily basis)

(Data expressed on daily basis)						
Animal number	Body wt. lb.	Food D.M. gm.	Faecal D.M. gm.	Metabolic faecal nitrogen		
				Total gm.	Per 100 gm. food D.M. gm.	Per 100 gm. faecal D.M. gm.
<i>Experiment 1</i>						
K1	276	1514	747	6.87	0.45	0.92
K2	264	1112	556	5.49	0.49	0.98
K3	276	1511	711	6.11	0.40	0.86
<i>Average</i>		1379			0.45	0.92
<i>Experiment 2</i>						
K1	275	1780	862	7.74	0.44	0.90
K2	242	1590	784	6.30	0.40	0.81
K3	270	1755	880	7.06	0.40	0.80
<i>Average</i>		1702			0.41	0.84
<i>Experiment 3</i>						
K1	244	1702	845	7.11	0.42	0.84
K2	240	1854	840	6.80	0.37	0.81
K3	280	2094	987	8.20	0.39	0.83
<i>Average</i>		1883			0.39	0.83

PART II

Effect of indigestible matter in food on the M. F. N. of cattle and rats

Mendel and Fine [1912] observed that the addition of agar-agar to a ration increased the faecal nitrogen excretion of dogs. Mitchell [1924] found that the nitrogen content of the faeces of rats was raised when they consumed filter paper along with a nitrogen-free diet. Adolf and Wu [1934], however recorded a decrease in the amount of nitrogen in the faeces (per 100 gm. food dry matter) when filter paper was incorporated in the diet of rats. They further observed that the faecal nitrogen of human was raised when dry cabbage fibre was included in the ration, but the reverse was true for the moist fibre. The work of Funnell *et al* [1936] on a human subject suggests that the value of M. F. N. increases when fibre (prepared from bran) is added to the diet. The observations of Duckworth and Godden [1941] on rats are also in agreement with those of Mitchell [1924].

The above-mentioned investigations have been conducted on animals which habitually ingest concentrated foods, with but small amounts of dietary fibre. It is not surprising that the available information regarding the effect of fibre on the M. F. N. of these animals is, of a fragmentary character. Although the diet of ruminants normally contains a high percentage of indigestible fibre, practically nothing is known on the effect of fibre on their M. F. N. Hutchinson and Morris [1936] carried out some preliminary experiments bearing upon this problem. They found that an increased excretion of M. F. N. resulted when paper-pulp was added to a fibre-free and 'nitrogen-free' ration of goats.

Some observations recorded by the present authors [Mukherjee and Kehar, 1948], however, suggested that the M. F. N. of cattle is markedly affected by the content of indigestible matter in food. Since no accurate information is available in literature on this important problem, a thorough study of the subject was undertaken. A comparative investigation was also carried out on rats, as the results of published experiments were controversial.

EXPERIMENTAL

(a) Experiments on cattle

Oat straw and a particular sample of Izatnagar hay were found to contain a very small proportion of indigestible matter; on the other hand, wheat straw was rich in indigestible fibre. Hence, for studying the effect of the level of indigestible matter in food on the M. F. N. of cattle, these roughages were conveniently utilized (Table II).

In experiment 4, three Haryana bullocks were fed Izatnagar hay containing 25 per cent indigestible dry matter (I. D. M.) and the M. F. N. data were compared with those of experiment 4 (a) where the same animals consumed low-N ration No. 1 (43 per cent I. D. M.) containing untreated wheat straw [Mukherjee and Kehar, 1946]. Similarly, in experiments 5 and 6, rations containing oat straw (26 per cent I. D. M.) and wheat straw (48 per cent I. D. M.) were respectively fed to local cattle. Adequate amounts of minerals were included in these rations; in addition, a salt (NaCl) lick was provided. The technique employed in these experiments was identical with that adopted in Part I of this paper.

TABLE II

Effect of indigestible matter in food on the M. F. N. of cattle

(Figures expressed on daily basis)

Animal number	Body wt. lb.	Food D.M. gm.	Faecal D.M. gm.	Metabolic faecal nitrogen			'Indigestible' matter in ration
				Total gm.	Per 100 gm. food D.M. gm.	Per 100 gm. faeca D.M. gm.	
<i>Experiment 4</i>							
Ha 11	1035	5837	1545	13.0	0.23	0.84	25
Ha 12	1010	5549	1377	11.9	0.22	0.86	
Ha 13	929	4875	1101	10.3	0.21	0.94	
<i>Average</i>					0.22	0.88	
<i>Experiment 4 (a)</i>							
Ha 11	979	5082	2047	17.8	0.35	0.87	43
Ha 12	895	4804	2224	17.6	0.36	0.79	
Ha 13	890	4754	2005	16.3	0.34	0.81	
<i>Average</i>					0.35	0.82	
<i>Experiment 5</i>							
L1	804	5849	1710	16.7	0.29	0.98	26
L5	812	5938	1538	15.8	0.27	1.03	
L6	776	5600	1201	12.6	0.22	1.05	
<i>Average</i>					0.26	1.02	
<i>Experiment 6</i>							
L1	824	3880	1856	16.0	0.41	0.86	48
L5	826	4000	2024	16.4	0.41	0.81	
L6	740	3670	1686	13.8	0.38	0.82	
<i>Average</i>					0.40	0.83	

Finally, the M. F. N. data of all the experiments recorded so far (in Parts III and IV of this series) were arranged separately for three types of cattle, in ascending order of the level of the faecal dry matter output (*vide* Tables III and IV). This was done with a view to gain further insight into the problem.

TABLE III

Influence of the level of faecal dry matter output on the M. F. N. of cattle

(Figures expressed on daily basis)

Serial number	Faecal D.M. gm.	Metabolic nitrogen			Ration
		Total gm.	Per 100 gm. food D.M. gm.	Per 100 gm. faecal D.M. gm.	
			<i>Hariana bullocks</i>		
1	885	7.0	0.20	0.86	'N-free'
2	935	8.5	0.26	0.91	do.
3	1101	10.3	0.21	0.94	Izatnagar hay
<i>Average</i>	974	8.8	0.22	0.90	
4	1276	13.2	0.26	1.03	'N-free'
5	1335	12.6	0.30	0.95	'Low-N' No. 2
6	1377	11.9	0.29	0.86	Izatnagar hay
<i>Average</i>	1329	12.6	0.26	0.95	
7	1400	12.3	0.32	0.88	'N-free'
8	1473	13.8	0.36	0.94	do.
9	1508	14.8	0.42	0.98	do.
<i>Average</i>	1460	13.6	0.37	0.93	
10	1545	12.9	0.23	0.84	Izatnagar hay
11	1718	14.0	0.34	0.82	'Low-N' No. 2
12	1823	14.8	0.40	0.81	'Low-N' No. 1
<i>Average</i>	1695	13.9	0.32	0.82	

TABLE III—*contd.*

Influence of the level of faecal dry matter output on the M. F. N. of cattle
(Figures expressed on daily basis)

Serial number	Faecal D.M. gm.	Metabolic nitrogen			Ration
		Total gm.	Per 100 gm. food D.M. gm.	Per 100 gm. faecal D.M. gm.	
			<i>Hariana bullocks</i>		
13	1860	15.8	0.42	0.84	'Low—N' No. 1
14	2005	16.3	0.34	0.81	do. No. 1
15	2020	16.7	0.30	0.83	do. No. 2
<i>Average</i>	1962	16.3	0.35	0.83	
16	2047	17.8	0.35	0.87	'Low—N' No. 1
17	2145	16.6	0.41	0.77	do.
18	2224	17.6	0.36	0.79	do.
<i>Average</i>	2130	17.3	0.37	0.81	

TABLE IV

Influence of the level of faecal dry matter output on the M. F. N. of cattle
(Figures expressed on daily basis)

Serial number	Faecal D.M. gm.	Metabolic nitrogen			Ration
		Total gm.	Per 100 gm. food D.M. gm.	Per 100 gm. faecal D.M. gm.	
			<i>Kumaun bullocks</i>		
1	556	5.49	0.49	0.98	Wheat straw & salts
2	711	6.11	0.40	0.86	do.
3	747	6.87	0.45	0.92	do.
<i>Average</i>	671	6.16	0.45	0.92	

TABLE IV—*contd.*

Influence of the level of faecal dry matter output on the M. F. N. of cattle
(Figures expressed on daily basis)

Serial number	Faecal D.M. gm.	Metabolic nitrogen			Ration
		Total gm.	Per 100 gm. food D.M. gm.	Per 100 gm. faecal D.M. gm.	
			<i>Kumaon bullocks</i>		
4	784	6.30	0.40	0.81	Wheat straw and salts
5	840	6.79	0.41	0.81	do.
6	845	7.11	0.42	0.84	do.
<i>Average</i>	823	6.73	0.41	0.82	
7	862	7.74	0.44	0.90	do.
8	880	7.06	0.36	0.80	do.
9	987	8.20	0.38	0.83	do.
<i>Average</i>	910	7.67	0.39	0.84	
			<i>Local bullocks</i>		
1	850	8.9	0.42	1.05	'Low-N' No. 1
2	896	8.4	0.37	0.92	'N-free'
3	983	9.8	0.36	1.00	'Low-N' No. 2
<i>Average</i>	910	9.0	0.38	0.99	
4	1044	9.3	0.32	0.90	'N-free'
5	1056	9.3	0.31	0.88	do.
6	1082	10.6	0.46	0.98	'Low-N' No. 1
<i>Average</i>	1061	9.7	0.36	0.92	

TABLE IV—*contd.**Influence of the level of faecal dry matter output on the M. F. N. of cattle*

(Figures expressed on daily basis)

Serial number	Faecal D.M. gm.	Metabolic nitrogen			Ration
		Total gm.	Per 100 gm. food D.M. gm.	Per 100 gm. faecal D.M. gm.	
			<i>Local bullocks</i>		
7	1165	10.3	0.43	0.88	'Low—N' No. 1
8	1201	12.5	0.22	1.05	Oat straw and salts
9	1202	10.3	0.42	0.87	'Low—N' No. 2
<i>Average</i>	1189	11.0	0.36	0.93	
10	1368	12.0	0.35	0.87	'Low—N' No. 2
11	1538	15.8	0.27	1.03	Oat straw and salts
12	1686	13.8	0.38	0.82	Wheat straw and salts
<i>Average</i>	1531	13.9	0.33	0.91	
13	1710	16.7	0.29	0.98	Oat straw and salts
14	1856	16.0	0.41	0.86	Wheat straw and salts
15	2024	16.4	0.41	0.81	do.
<i>Average</i>	1863	16.4	0.37	0.83	

(b) *Experiments on rats*

Six male albino rats weighing 190 to 225 gm. were used in experiments 7 and 8. They were selected from a colony of homozygous animals bred in the laboratory. In experiment 7, they were offered a low-nitrogen fibre-free ration containing ether extracted whole egg, dried below 60°C. After an interval of one week on a normal stock diet the same rats were fed in experiment 8 a high-fibre diet which differed from the above mentioned ration only in the fibre content. Each rat was also administered 10 micrograms of thiamine daily with food. Other vitamins were

not added, since the experiments were of short duration and the diet contained egg as one of the ingredients. Ferric oxide and barium sulphate were used as faeces markers. The indicator mentioned first was fed to the rats on the last day of the pre-experimental and collection periods, while the latter was used on other days. Ferric oxide was found to be more satisfactory for the purpose of demarcating the faeces between different periods than chromic oxide. The adjustment and collection periods were respectively five and seven days in length. The fibre was prepared from ground wheat straw by (1) boiling the straw with dilute (1.25 per cent) solution of sulphuric acid for $\frac{1}{2}$ hour, (2) washing with hot water to free it from acid, (3) boiling with dilute (1.25 per cent) caustic soda solution for $\frac{1}{2}$ hour, (4) washing with hot water to remove the alkali and finally (5) drying in the oven at 100 to 105°C. The fibre isolated in this way, contained only a negligible amount of nitrogen (0.04 per cent N on dry basis).

The rats were kept in individual metabolism cages made of galvanized iron sheets and wire-netting. Tap water was supplied in the cages in small glass bottles fitted with corks and nozzles. The ration was offered to the rats in non-scatter food cups similar to that devised by McCollum and Simmonds [1929]. With this arrangement there was practically no scattering of food when the latter was moistened with a small quantity of water to make the particles adhere to one another. The cages were placed on glass funnels (diameter 12 in) with short stem. The funnels were fixed over a wooden stand so that the stem remained in vertical position. For the quantitative separation of urine and faeces, a device similar to that used by Ackroyd and Hopkins [1916] was employed. The small conical flasks (capacity 150 c.c.), in which urine was collected, were kept inside a large beaker (capacity 1,000 c.c.) and directly under the stem of the funnel. Since the value for the endogenous urinary nitrogen of the rats was required for another purpose, 1 c.c. of 25 per cent sulphuric acid was kept in the conical flasks every day in order to prevent loss of nitrogen. A few drops of a 10 per cent alcoholic solution of thymol were also placed daily at the bottom of the beakers for protecting the faeces from bacterial decomposition. Every twenty-four hours (during the collection period only), the urine and faeces were taken out and the funnel, bulb, etc., washed with small quantities of dilute (1 per cent) sulphuric acid and water; the washings were added to the urine.

The faeces of the rats were transferred to individual Petri dishes and particles of food and semen (if any), as well as adhering hair, were removed by mechanical means. The intact pellets of faeces were then washed with small quantities of a hot (about 80°C.) solution of dilute (2 per cent) acetic acid to free them from possible contamination with urine, the washings being added to the urine. The faeces were then dried over a boiling water bath. The residues of individual animals, together with a few food particles which would occasionally fall on the funnel were daily removed to Petri dishes and dried partially by placing in an oven for an hour or two. This routine was followed every day during the collection period. At the end of the experiment, the faeces and residue, corresponding to the experimental period were completely dried in the oven and weighed. The dried faeces were then ground in a glass mortar and the hair was carefully separated by sifting [Schneider, 1935]; total nitrogen was determined in the hair-free faeces. The dry matter and nitrogen content of a representative sample of the ration was also determined at the same time.

RESULTS AND DISCUSSION

(a) Experiments on cattle

The data of experiments 4, 4 (a), 5 and 6, which were obtained with two types of cattle, are presented in Table II. It shows that the total excretion of metabolic nitrogen, as well as M. F. N. expressed per 100 gm. of food dry matter, were markedly raised when rations containing a high content of indigestible matter were fed. The rise in the value of M. F. N. nearly corresponded to the increase in the percentage of indigestible matter in the diet. On the other hand, the M. F. N., when expressed in terms of faecal dry matter output, was lowered under the same conditions.

The pooled data of all the experiments including those of the preceding paper, are arranged in Tables III and IV. A reference to these two tables and to figs. 3, 4 and 5 (reproduced in the next article pp. 37-39) leads to the conclusion that when the level of faecal dry matter output increases (a) total M. F. N. steadily rises, and (b) M. F. N., when stated on faecal dry matter basis, decreases almost consistently, while (c) in the case of M. F. N. figures expressed in terms of food dry matter, no definite trend is noticed.

(b) Experiments on rats

The composition of the fibre-free and high-fibre rations for rats is recorded in Table V.

TABLE V

Composition of low-N diets for rats

Ingredients	High-fibre ration	Fibre-free ration
Dried ether-extracted whole egg	5 parts	5 parts
Salt mixture (Hubbel, Mendel and Wakeman)	2 parts	2 parts
Butter fat	8 parts	8 parts
Cod liver oil	2 parts	2 parts
Sugar	10 parts	10 parts
Sodium chloride	1 part	1 part
Starch	55 parts	70 parts
Marker (Fe_2O_3 or Ba SO_4)	2 parts	2 parts
Purified fibre	15 parts	0 parts
Total	100 parts	100 parts

TABLE VI

Effect of dietary fibre on the M. F. N. of rats
(Data expressed on daily basis)

Rat number	Body weight gm.	Food D.M. gm.	Faecal D.M. gm.	Metabolic faecal nitrogen		Indigestible matter in ration per cent
				Per 100 gm. food D.M. gm.	Per 100 gm. faecal D.M. gm.	
Experiment 7 (fibre-free ration)						
1	215	8.98	0.77	0.18	2.15	9
2	223	8.95	0.77	0.19	2.22	
3	213	9.07	0.71	0.17	2.20	
4	203	8.55	0.82	0.21	2.15	
5	206	8.95	0.68	0.18	2.42	
6	212	9.10	0.89	0.17	1.72	
Average	212	8.92	0.77	0.18	2.14	9
C. V.				7.4	13.6	
Experiment 8 (high-fibre ration)						
1	190	9.10	1.83	0.23	1.17	20
2	218	9.15	1.91	0.25	1.18	
3	205	9.15	1.81	0.23	1.16	
4	195	9.13	1.90	0.24	1.17	
5	225	9.16	1.86	0.26	1.27	
6	215	9.13	1.87	0.25	1.20	
Average	208	9.14	1.86	0.24	1.19	20
C. V.				3.9	4.1	

The observations on rats (experiments 7 and 8) are presented in Table VI. This table shows that the food intakes on both rations were identical. It also indicates that the M. F. N. of rats (calculated in the traditional way, i.e., on food dry matter basis) increases with a rise in the percentage of indigestible fibre in the ration. This observation is in agreement with that of Mitchell [1924]. Under similar conditions, the M. F. N. figure expressed on faecal dry matter basis decreases. These

RESULTS AND DISCUSSION

(a) Experiments on cattle

The data of experiments 4, 4 (a), 5 and 6, which were obtained with two types of cattle, are presented in Table II. It shows that the total excretion of metabolic nitrogen, as well as M. F. N. expressed per 100 gm. of food dry matter, were markedly raised when rations containing a high content of indigestible matter were fed. The rise in the value of M. F. N. nearly corresponded to the increase in the percentage of indigestible matter in the diet. On the other hand, the M. F. N., when expressed in terms of faecal dry matter output, was lowered under the same conditions.

The pooled data of all the experiments including those of the preceding paper, are arranged in Tables III and IV. A reference to these two tables and to figs. 3, 4 and 5 (reproduced in the next article pp. 37-39) leads to the conclusion that when the level of faecal dry matter output increases (a) total M. F. N. steadily rises, and (b) M. F. N., when stated on faecal dry matter basis, decreases almost consistently, while (c) in the case of M. F. N. figures expressed in terms of food dry matter, no definite trend is noticed.

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The composition of the fibre-free and high-fibre rations for rats is recorded in Table V.

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Ingredients	High-fibre ration	Fibre-free ration
Dried ether-extracted whole egg	5 parts	5 parts
Salt mixture (Hubbel, Mendel and Wakeman)	2 parts	2 parts
Butter fat	8 parts	8 parts
Cod liver oil	2 parts	2 parts
Sugar	10 parts	10 parts
Sodium chloride	1 part	1 part
Starch	55 parts	70 parts
Marker (Fe_2O_3 or BaSO_4)	2 parts	2 parts
Purified fibre	15 parts	0 parts
Total	100 parts	100 parts

TABLE VI

Effect of dietary fibre on the M. F. N. of rats

(Data expressed on daily basis)

Rat number	Body weight gm.	Food D.M. gm.	Faecal D.M. gm.	Metabolic faecal nitrogen		'Indigestible' matter in ration per cent
				Per 100 gm. food D.M. gm.	Per 100 gm. faecal D.M. gm.	
<i>Experiment 7 (fibre-free ration)</i>						
1	215	8.98	0.77	0.18	2.15	9
2	223	8.95	0.77	0.19	2.22	
3	213	9.07	0.71	0.17	2.20	
4	203	8.55	0.82	0.21	2.15	
5	206	8.95	0.68	0.18	2.42	
6	212	9.10	0.89	0.17	1.72	
<i>Average</i>	212	8.92	0.77	0.18	2.14	}
C. V.				7.4	13.6	
<i>Experiment 8 (high-fibre ration)</i>						
1	190	9.10	1.83	0.23	1.17	20
2	218	9.15	1.91	0.25	1.18	
3	205	9.15	1.81	0.23	1.16	
4	195	9.13	1.90	0.24	1.17	
5	225	9.16	1.86	0.26	1.27	
6	215	9.13	1.87	0.25	1.20	
<i>Average</i>	208	9.14	1.86	0.24	1.19	}
C. V.				3.9	4.1	

The observations on rats (experiments 7 and 8) are presented in Table VI. This table shows that the food intakes on both rations were identical. It also indicates that the M. F. N. of rats (calculated in the traditional way, i.e., on food dry matter basis) increases with a rise in the percentage of indigestible fibre in the ration. This observation is in agreement with that of Mitchell [1924]. Under similar conditions, the M. F. N. figure expressed on faecal dry matter basis decreases. These

findings are in line with our results obtained on cattle (cf. Table II). It is interesting to note (cf. Tables II and VI) that the M. F. N. figures of cattle and rats on rations containing comparable amounts of indigestible matter are nearly alike. Incidentally, the observations of rats (cf. Table VI) afford an opportunity of examining the applicability of the present authors' method of expressing the M. F. N. When the coefficients of variation (C. V.) of the individual M. F. N. data of rats are considered, it is found that the new method of expressing M. F. N. gives consistent results (C.V.=4.1 per cent) when the ration is fairly high in indigestible fibre. With the fibre-free diet, however, there is closer agreement between the individual figures when they are stated on food dry matter basis; nevertheless, in this case too the authors' procedure of expressing M. F. N. can be adopted without appreciable error (cf. C.V.=13.6 per cent). These facts, therefore, lend further support to the proposed method.

SUMMARY AND CONCLUSIONS

The effect of two factors, namely the level of food consumption and the amount of indigestible matter in the ration, on the M. F. N. of cattle and rats was studied.

The M. F. N. of cattle, expressed either in terms of food intake or faecal dry matter output, was found to decrease with an increase in the level of food intake.

In cattle, as well as rats, the M. F. N. per 100 gm. food dry matter was markedly raised when rations rich in indigestible matter were fed. Under the same circumstances the M. F. N. figures calculated on faecal dry matter basis were lowered.

The proposed method of expressing M. F. N. in terms of faecal dry matter output was applicable to rats as well as cattle.

In cattle, when the level of faecal dry matter excretion increased (*a*) total M. F. N. steadily rose and (*b*) M. F. N. (per 100 gm. faecal dry matter) consistently decreased. No definite trend could, however, be noticed in the variation of M. F. N. figures calculated in the traditional way i.e., in terms of food dry matter consumed.

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STUDIES ON PROTEIN METABOLISM

V. A GRAPHICAL METHOD FOR DETERMINING THE METABOLIC FAECAL NITROGEN OF CATTLE

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(With five text figures)

THE two preceding papers of this series [Mukherjee and Kehar, 1948 and Kehar and Mukherjee, 1948] were concerned with (a) improvements in the technique of estimating the metabolic faecal nitrogen (M. F. N.) and (b) effect of certain factors on the excretion of M. F. N. The observations recorded therein suggested that M. F. N. of cattle depended more on the level of faecal dry matter output than on any other factor. It led us to consider if a knowledge of the quantitative relationship between these two variables might prove useful in predicting the M. F. N. when the dry matter excretion in the faeces is known. A statistical treatment of the available data was, therefore, undertaken with a view to (i) explore the possibility of evolving a graphical method of estimating M. F. N. of cattle and (ii) interpret the data, so as to yield further information.

METHODS AND MATERIALS

The original data were obtained in experiments which have already been recorded (Mukherjee and Kehar, *loc cit*). These data are summarized in Tables I and II for convenience of reference.

TABLE I

Summary of the data on Haryana bullocks

Experiment number	Ration	Body weight lb.	Faecal dry matter = F.D.M. gm.	Metabolic faecal N	
				Total gm.	Per 100 gm. F.D.M. gm.
1	'N-free'	1180	1276	13.2	1.03
1	do.	894	1473	13.8	0.94
1	do.	863	1400	12.3	0.88
1(a)	do.	924	935	8.5	0.91
1(a)	do.	878	885	7.6	0.86
1(a)	do.	839	1508	14.8	0.98

TABLE I—*contd.**Summary of the data on Hariana bullocks—contd.*

Experiment number	Ration	Body weight lb.	Faecal dry matter = F.D.M. gm.	Metabolic faecal N	
				Total gm.	Per 100 gm. F.D.M. gm.
2	Low-N No. 1	1210	1860	15.8	0.85
2	do.	946	1823	14.8	0.80
2	do.	947	2145	16.6	0.78
2(a)	do.	979	2047	17.8	0.87
2(a)	do.	890	2005	16.3	0.81
2(a)	do.	895	2224	17.6	0.79
3	Low-N No. 2	1186	1335	12.6	0.95
3	do.	887	1718	14.8	0.81
3	do.	895	2020	16.7	0.83
9	Izatnagar hay	929	1101	10.3	0.93
9	do.	1010	1377	11.9	0.98
9	do.	1035	1545	12.9	0.84

TABLE II

Summary of the data on local and Kumaun bullocks

Experiment number	Ration	Body weight lb.	Faecal dry matter = F.D.M. gm.	Metabolic faecal N	
				Total gm.	Per 100 gm. F.D.M. gm.
		<i>Local bullocks</i>			
4	'N-free'	516	896	8.4	0.94
4	do.	588	1056	9.3	0.88
4	do.	560	1044	9.3	0.90
5	Low-N No. 1	536	850	8.9	1.05
5	do.	570	1082	10.6	0.98

TABLE II—*contd.**Summary of the data on local and Kumaun bullocks—contd.*

Experiment number	Ration	Body weight lb.	Faecal dry matter = F.D.M. gm.	Metabolic faecal N	
				Total gm.	Per 100 gm. F.D.M. gm.
Local bullocks					
5	Low-N No. 1	610	1165	10.3	0.88
6	Low-N No. 2	570	983	9.8	1.00
6	do.	540	1202	10.3	0.86
6	do.	630	1363	12.0	0.87
14	Wheat straw and salts	824	1856	16.0	0.87
14	do.	826	2024	16.4	0.81
14	do.	740	1686	13.8	0.82
15	Oat straw and salts	776	1201	12.5	1.05
15	do.	812	1538	15.8	1.03
15	do.	804	1710	16.7	0.98
Kumaun bullocks					
11	Wheat straw and salts	264	556	5.5	0.99
11	do.	276	711	6.1	0.86
11	do.	276	747	6.9	0.91
12	do.	275	862	7.7	0.90
12	do.	280	880	7.1	0.80
12	do.	242	784	6.3	0.80
13	do.	244	845	7.1	0.84
13	do.	280	987	8.2	0.83
13	do.	240	840	6.8	0.80

The results of the statistical analysis are presented in Table III and represented graphically in Figs. 1 and 2.

TABLE III

Correlation coefficients and regression equations relating daily faecal dry-matter and faecal nitrogen

Serial number	Figures correlated*		Type of cattle**	No. of observations	Correlation Coefficient	Value of 't'		Remarks***	Regression equations y =
	X	Y				Found	Theoretical Found at 1 per cent level		
A)	F.D.M. (gm.)	M.F.N. (gm.)	K	9	(A) 0.918	0.13	3.60	H.S.	1.800 + 0.00619X
	do.	do.	Ha	18	0.975	17.60	2.92	do.	2.486 + 0.00707X
	do.	do.	L	15	0.941	10.00	3.01	do.	1.960 + 0.00708X
(B)	F.D.M. (gm.) (B.W.) 0.73	M.F.N. (gm.) (B.W.) 0.73	K	9	(B) 0.925	0.45	3.50	do.	0.0820 + 0.00589X
	do.	do.	Ha	18	0.862	6.80	2.92	do.	0.02561 + 0.00620X
	do.	do.	L	15	0.853	5.89	3.01	do.	0.03876 + 0.00577X
(C)	F.D.M. (gm.) (B.W.) 0.73	N in F.D.M. (per cent)	K	9	(C) -0.802	3.55	3.50	do.	1.1791 - 0.0232X
	do.	do.	Ha	18	-0.659	3.50	2.92	do.	1.0424 - 0.0158X
	do.	do.	L	15	-0.674	3.20	3.01	do.	1.2447 - 0.0281X

*F.D.M.—Faecal dry matter output; B.W.—av. body-weight of animals during expt. in lb.

**K.—Kumaon; Ha—Hariana and L—Local bullocks

***H.S.—Highly significant

In order to deal with the data of animals differing widely in body-weight, some computations were made by reducing one or both variables (arbitrarily) on the basis of 0.73 power of the respective body-weights.

RESULTS AND DISCUSSION

(a) Choice of the method

Table III and Fig. 1 show that there is a linear relationship between the faecal dry matter and the metabolic faecal nitrogen data in the case of all the three types of cattle studied and the values of r , viz., 0.92, 0.97 and 0.94 (cf. under A in Table III) indicate that the correlation is highly significant. In fact, the correlation was closest between these two sets of variables. The correlation was not improved by reducing both these variables on the basis of 0.73 power of the body-weight. This is shown by the lower values of r , viz., 0.92, 0.86 and 0.85 (cf. under B in Table III). In both cases, however, the correlation was highly significant. The graphs included in Figs. 1 and 2 have been drawn by making use of the regression equations given in the last column of Table III. The high value of r indicates that the M. F. N. of cattle can be estimated graphically with a fair degree of precision, if the faecal dry

matter output is known. For this purpose, a reduction of the variables on the basis of 0.73 power of body weight offered no advantage. Precision suffered to a slightly greater extent when the metabolic nitrogen was estimated by using the equations grouped under C in Table III. Hence, Figs. 1 and 2 (particularly the former) can be recommended for the graphical determination of metabolic faecal nitrogen of cattle. In this method, the average daily output of faecal dry matter has to be estimated experimentally.

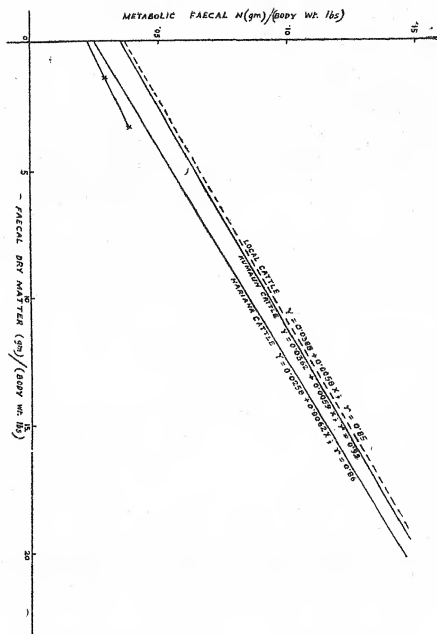


FIG. 1. Relation between metabolic faecal nitrogen and level of faecal dry matter output (on daily basis) of cattle and rats

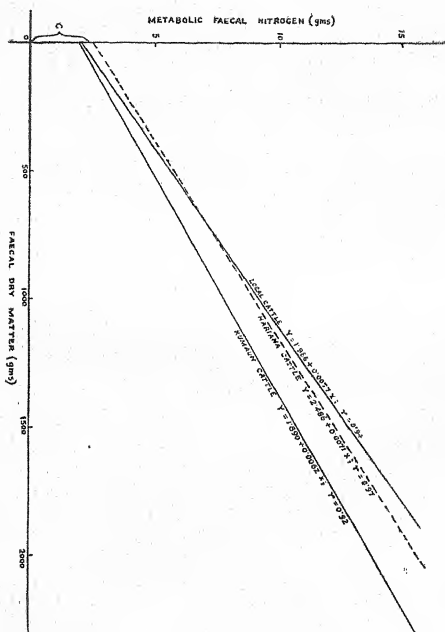


Fig. 2. Relation between metabolic faecal nitrogen and faecal dry matter output (on daily basis) of three types of cattle

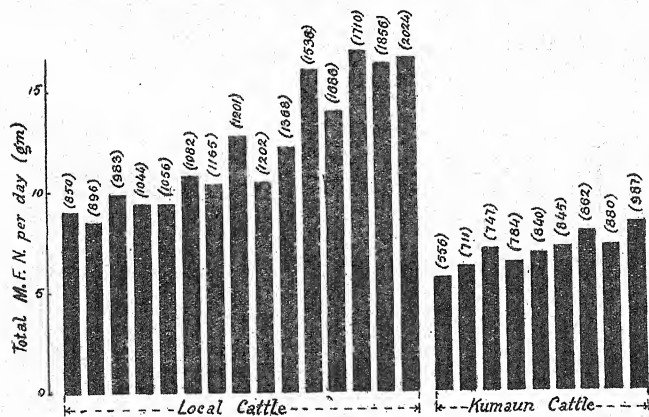
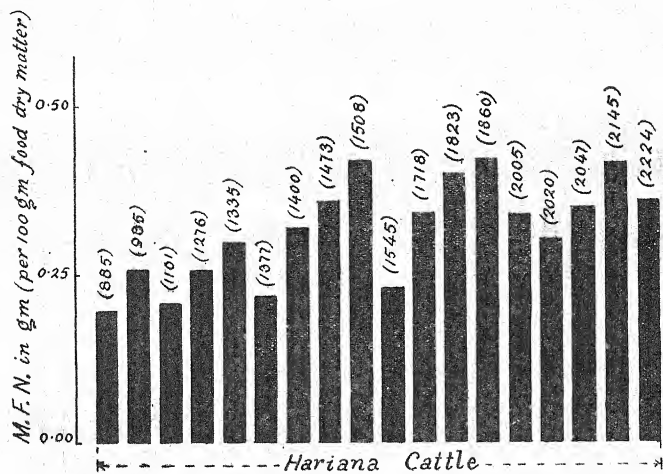


FIG. 3. The total metabolic faecal nitrogen of cattle at increasing levels faecal dry matter output.
 [NOTE.—The figures in parentheses give daily output of faecal dry matter (gm.)]

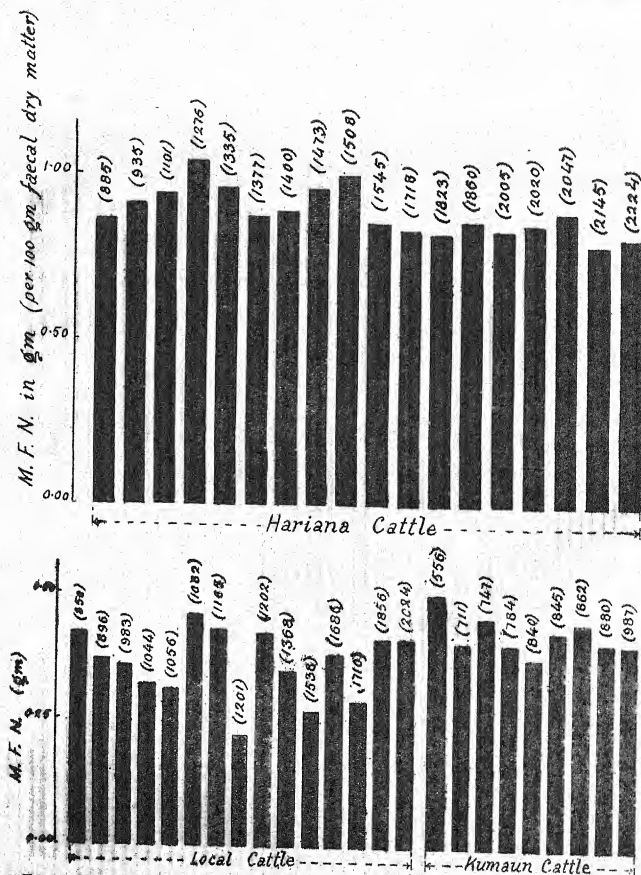


FIG. 4. The metabolic faecal nitrogen (expressed on food dry matter basis) of cattle at increasing levels of faecal dry matter output.

[NOTE.—The figures in parentheses give the daily output of faecal dry matter (gm.)]

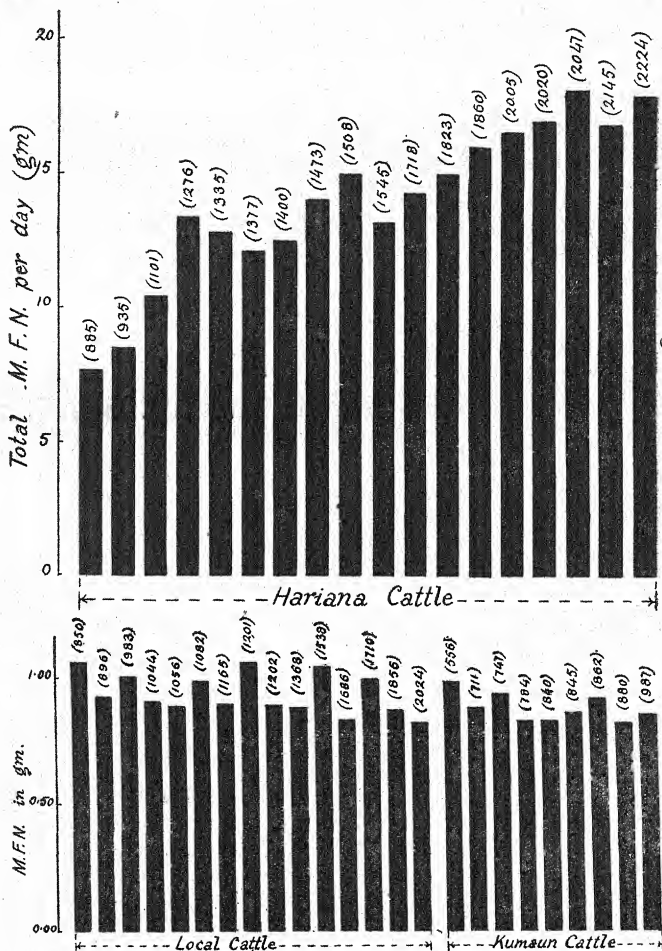


FIG. 5. The metabolic faecal nitrogen (expressed on faecal dry matter basis) of cattle at increasing levels of faecal dry matter output
 [Norm.—Faecal dry matter output given in parentheses]

Although good linear relationship has been found for faecal dry matter and faecal nitrogen (both with and without reference to body weight raised to 0.73 power), it is necessary to point out here that this conclusion is strictly applicable to the range within which observations were actually made; extrapolation far beyond the experimentally determined points may not, therefore, yield accurate information. In other words, the predicted values of M. F. N. will be highly accurate, only when the level of faecal dry matter output is sufficiently near the range of observations. Nevertheless, the range of usefulness can be easily widened by estimating the faecal nitrogen output at required levels of faecal dry matter output, using the simplified method developed by Mukherjee and Kehar [1948].

An application of the graphical method proposed above will, doubtless, be very useful in connection with investigations relating to the true digestibility and biological value of the proteins of cattle feeds according to the Thomas-Mitchell method, Mitchell [1924].

(b) *Effect of species and breed on M. F. N.*

In Fig. 2 the data obtained in our experiments on rats [cf. Kehar and Mukherjee, 1948] are also represented. It shows that the graph corresponding to the observation on rats is very similar to those for cattle. This suggests that the laws governing the excretion of M. F. N. of rats and cattle are essentially alike. The metabolic nitrogen of cattle is comparatively high, mainly because they normally consume (and excrete) large amount of indigestible matter.

Fig. 2 also shows that the graphs for the three types of cattle differ to a small extent. This difference does not seem to be significant, because the observations on the three types of bullocks were not made in identical experiments. The Kumaun animals were fed a diet of constant composition in all the experiments, while in the case of Haryana and local cattle, the rations differed widely in character. Further the body weight of the three types of animals were widely different (Kumaun cattle, 240 to 280 lb.; Haryana cattle, 839 to 1,210 lb.; local cattle, 516 to 826 lb.). A consideration of these facts leads to the conclusion that breed characteristics do not affect the M. F. N. of cattle appreciably.

(c) *Constant fraction of the M. F. N.*

Referring again to Figs. 1 and 2 it is seen that the straight lines representing the regression equations cut the y-axis on extrapolation. The y-intercept (marked C in the charts) may be called the constant fraction of the M. F. N. according to the concept of Schneider [1935]. Schneider [1934, 1935], however, established the existence of this fraction in the case of rats and swine only. The above-mentioned figures suggest that the constant fraction of M.F.N. is a small part of the total M. F. N. when cattle consume an adequate amount of food. On comparing the y-intercepts of cattle and rats in Fig. 2 it appears that the constant fraction is a comparatively large fraction of the total M. F. N. of rats since the latter consumes rations which are low in indigestible fibre. It must be emphasized at this point that the value of the constant fraction cannot be estimated accurately from these figures, since the range of observations from which the graphs were plotted did not include very low levels of faecal dry matter output.

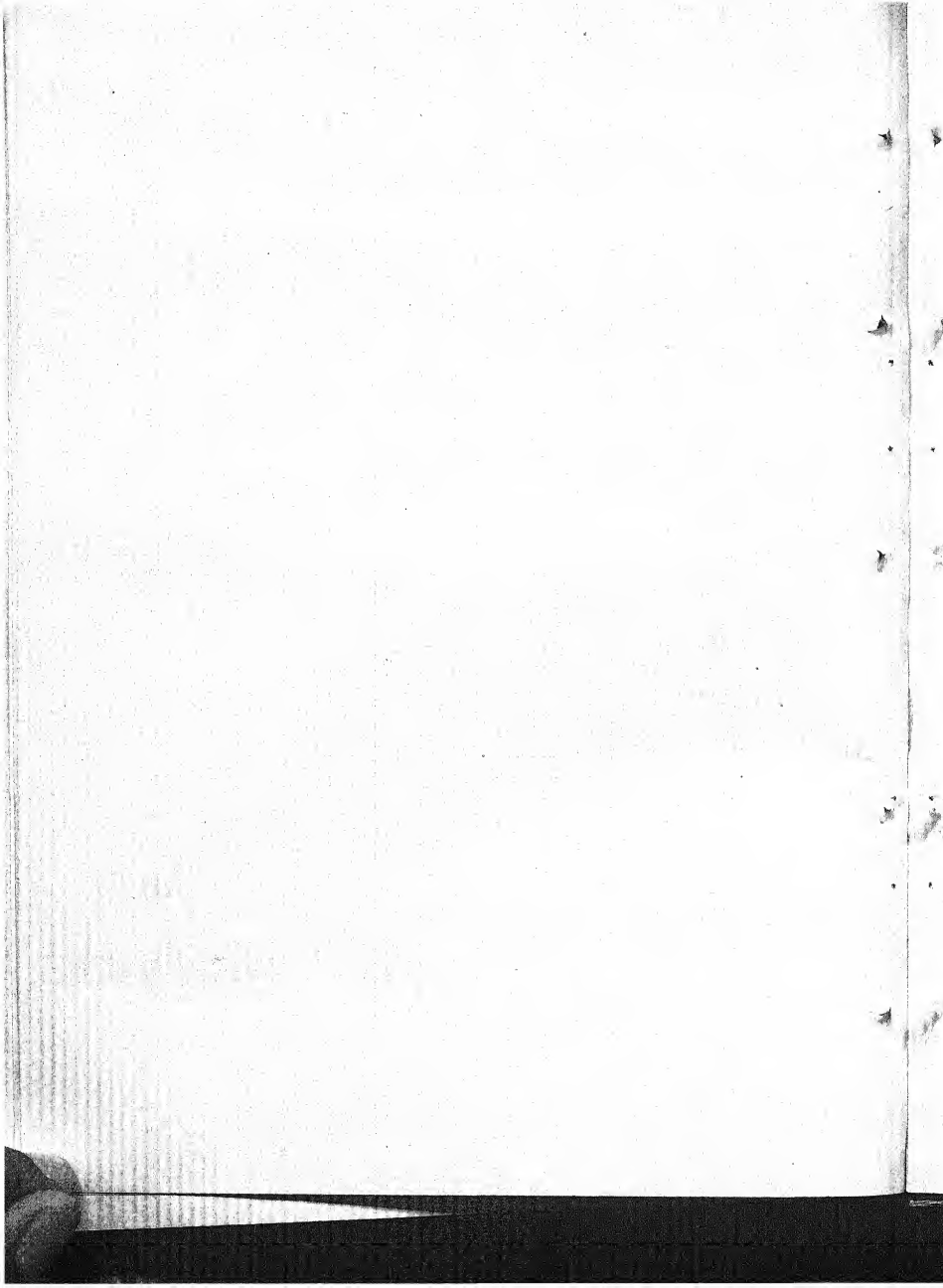
In contrast with the above findings, Hutchinson and Morris [1936] concluded from experiments on goats and sheep that the constant fraction of the M. F. N. of these animals was a fairly large proportion of the total M. F. N. A critical examination of their graph, however, shows that the scatter of the plotted data was too high to warrant a definite conclusion regarding the magnitude of the constant fraction. Further, it was implied in their argument that the faecal nitrogen output of these animals under fasting condition corresponded to an intake of food which was practically zero. This assumption, however, cannot be justified by other available facts. Mendel and Fine [1912], on the basis of their work on non-ruminants, stated that, 'fasting faeces are, in great part derived from retained faecal matter resulting from food immediately preceding the period of inanition.' Observations of the present authors on fasting cattle (unpublished work) afford evidence that the faecal nitrogen excretion during the first nine days of fast corresponds, as it were, to a progressively lower intake of food.

SUMMARY AND CONCLUSIONS

Statistical analysis of 42 metabolic nitrogen data, which have already been published by the present authors, is reported here. The existence of a highly significant linear relationship between the level of faecal dry matter output and the metabolic faecal nitrogen of cattle was established as a result of this study and a graphical method of estimating M. F. N. of cattle was thus developed. A comparison of the graphs for three types of cattle suggested that breed characteristics do not affect the M. F. N. of cattle appreciably. Evidence was also obtained for the existence of a constant fraction of metabolic nitrogen in the case of cattle as in rats.

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DIGESTIBILITY AND NUTRITIVE VALUE OF *USAR* GRASS-HAY (*SPOROBOLUS ARABICUS*)

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THE digestibility and the nutritive value of *usar* grass-hay have been investigated under a scheme of the I. C. A. R. to assess the feeding value of indigenous grasses. *Usar* grass as the name indicates, grows on a 'usar' or barren land in which the permeability of water is hindered by the formation of alkali-salt hardpan a few inches below the surface. The soil moreover, contains excess of sodium carbonate and sodium sulphate. The hay used in the present investigation represents the October cut of the grass and was received through the courtesy of the Forest Department, U. P. Government.

Chemical composition of *usar* grass-hay

The chemical composition, together with that of some other varieties of indigenous grass-hays [Sen, 1938] is given in Table I.

TABLE I

Percentage composition on dry basis of *usar* and of some cultivated and indigenous grass hays

Name of grass	Organic constituents						Inorganic constituents		
	Total organic matter	Crude protein	Ether extract	Crude fibre	N.F.E.	Total carbohydrates	Total ash	CaO	P ₂ O ₅
<i>Usar (Sporobolus arabicus)</i>	91.16	6.12	1.02	34.39	84.02	8.84	0.30	0.30	0.33
<i>Anjan (Pennisetum centrichoides)</i>	89.82	4.87	0.83	32.91	51.21	84.12	10.18	0.36	0.72
<i>Dhub (Cynodon dactylon)</i>	87.46	11.10	1.38	18.38	56.60	74.96	12.54	0.49	0.60
<i>Rhodes (Chloris gayana)</i>	90.92	6.36	1.16	30.31	44.09	83.40	9.08	0.42	0.23
<i>Spear (Andropogon contortus)</i>	90.97	2.97	0.96	38.32	48.75	87.07	9.03	0.39	0.18

It is seen that *usar* grass-hay compares fairly well with that of Rhodes grass and some important minerals like lime and phosphate, in *usar* are quite normal for a hay. Due perhaps to the soil in which it grows, a peculiarity of the hay is that it contains a large amount of sodium and sulphur. From a number of analyses it has been found that usually the sulphur and soda contents lie between 0.6–0.8 and 1.5–2.2 per cent respectively.

Digestibility and nutritive value

Six adult Kumauni bullocks of an average weight of 241 lb. were used for feeding experiment which lasted for 30 days, the first 18 days constituting the preliminary feeding period. For remaining 12 days, collection bags were fitted on the animals and after allowing two days to get the animals used to the bags, twenty four hourly collections of excreta were made during the last ten days. Although the animals maintained a general healthy appearance during the feeding period they suffered on the average three per cent decrease in weight. The ration of the experimental animals was made up of (i) a scheduled quantity of rape-cake to meet the digestible protein requirement plus half-an-ounce of common salt, and (ii) *usar* grass *ad libitum*.

The daily residues of *usar* grass during the experimental period of ten days were collected separately for the individual animals and accounted for. In Table II is given the chemical composition of the feeding stuffs used.

TABLE II

Chemical composition on dry basis of the feeding stuffs

	Organic constituents						Inorganic constituents		
	Total organic matter	Crude protein	Ether extract	Crude fibre	N.F.E.	Total carbohydrates	Total ash	CaO	P ₂ O ₅
<i>Usar</i> hay	91.16	6.12	1.02	34.39	49.63	34.02	4.84	0.30	0.33
Rape-cake	89.85	36.61	9.44	8.81	34.99	43.80	10.15	1.17	2.72

In Table III is shown the food consumption including the average daily dry matter consumption of the hay per 100 lb. body weight.

TABLE III

Average daily consumption on dry basis of feeds

Animal number	Average live weight during the experiment lb.	Consumption			
		<i>Usar</i> grass gm.	Rape-cake gm.	Total gm.	<i>Usar</i> grass per 100 lbs. live weight gm.
470	250	2343	197.6	2540.6	937
465	220	1881	197.6	2078.6	822
440	236	2139	197.6	2336.6	907
417	264	2358	197.6	2555.6	893
374	233	1972	197.6	2169.6	846
359	210	1908	197.6	2105.6	909
<i>Average</i>	237	2100.2	197.6	2297.8	886

The hay was consumed at the rate of 886 gm. per 100 lb. body weight which shows, that it was fairly palatable. The digestibility of *usar* grass was determined by eliminating the nutrients digested from the rape-cake the digestibility co-efficients of which were obtained from data by Sen [1938].

The details of digestibility co-efficients for different co-nstituents of *usar* grass are worked out in Table IV.

TABLE IV

Digestibility co-efficients

	Crude protein	Ether extract	Crude fibre	N.F.E.	Total carbohydrates
	<i>Animal No. 470</i>				
Consumed from <i>usar</i>	143.4	23.9	805.7	1163.0	1968.7
Consumed from cake	72.3	18.7	17.4	69.1	86.5
<i>Total consumed</i>	215.7	42.6	823.1	1232.1	2055.2

TABLE IV—*contd.*

	Crude protein	Ether extract	Crude fibre	N.F.E.	Total carbo- hydrates
Excreted in faeces	108.9	18.1	451.6	855.0	1306.6
<i>Total digested</i>	106.8	24.5	371.1	377.1	748.6
Digested from cake	61.5	17.4	7.7	51.3	59.0
Digested from <i>usar</i>	45.3	7.1	363.8	325.8	698.6
Digestibility co-efficients	31.6	29.7	45.1	28.0	35.0
<i>Animal No. 165</i>					
Consumed from <i>usar</i>	115.1	19.9	646.9	933.5	1580.0
Consumed from cake	72.3	18.7	17.4	69.1	186.5
<i>Total consumed</i>	187.4	38.6	664.3	1002.6	1666.9
Excreted in faeces	77.0	12.1	289.1	544.9	834.0
<i>Total digested</i>	110.4	26.5	375.2	457.7	832.9
Digested from cake	61.5	17.4	7.7	51.3	59.0
Digested from <i>usar</i>	48.9	9.1	367.5	406.4	773.9
Digestibility co-efficients	42.5	46.4	56.8	43.5	149.0
<i>Animal No. 149.</i>					
Consumed from <i>usar</i>	130.9	21.8	735.5	1061.5	1797.0
Consumed from cake	72.3	18.7	17.4	69.1	86.5
<i>Total consumed</i>	203.2	40.5	752.9	1130.6	1883.5

TABLE IV—*contd.*

	Crude protein	Ether extract	Crude fibre	N.F.E.	Total carbohydrates
Excreted in faeces	87.1	15.1	386.6	717.8	1104.4
<i>Total digested</i>	116.1	25.4	366.3	412.8	779.1
Digested from cake	61.5	17.4	7.7	51.3	59.0
Digested from <i>usar</i>	54.6	8.0	358.6	361.5	720.1
Digestibility co-efficients	41.7	35.0	48.8	34.1	40.1
<i>Animal No. 417</i>					
Consumed from <i>usar</i>	144.3	24.1	810.9	1170.3	1981.2
Consumed from cake	72.3	18.7	17.4	69.1	86.5
<i>Total consumed</i>	216.6	42.8	828.3	1239.4	2067.7
Excreted in faeces	95.5	14.2	415.2	759.5	1174.7
<i>Total digested</i>	121.1	28.6	413.1	479.9	803.0
Digested from cake	61.5	17.4	7.7	51.3	59.0
Digested from <i>usar</i>	59.6	11.2	405.4	428.6	834.0
Digestibility co-efficients	41.3	46.6	50.0	36.6	42.1
<i>Animal No. 374</i>					
Consumed from <i>usar</i>	120.7	20.1	678.2	978.7	1656.9
Consumed from cake	72.3	18.7	17.4	69.1	86.5
<i>Total consumed</i>	193.0	38.8	695.6	1047.8	1743.4

TABLE IV—*conold.*

	Crude protein	Ether extract	Crude fibre	N.F.E.	Total carbo- hydrates
Excreted in faeces	74.0	13.3	322.2	611.7	933.9
<i>Total digested</i>	119.0	25.5	373.4	436.1	809.5
Digested from cake	61.5	17.4	7.7	51.3	59.0
Digested from <i>usar</i>	57.5	8.1	365.7	384.8	750.5
Digestibility co-efficients	47.6	40.3	53.9	39.3	45.3
<i>Animal No. 359</i>					
Consumed from <i>usar</i>	116.8	19.5	656.1	946.9	1603.0
Consumed from cake	72.3	18.7	17.4	69.1	86.5
<i>Total consumed</i>	189.1	38.2	673.5	1016.0	1689.5
Excreted in faeces	75.0	11.0	302.7	585.8	888.5
<i>Total digested</i>	114.1	27.2	370.8	430.2	801.0
Digested from cake	61.5	17.4	7.7	51.3	59.0
Digested from <i>usar</i>	52.6	9.8	363.1	378.9	742.0
Digestibility co-efficients	45.1	50.4	55.3	40.0	46.3

For comparison, the percentage composition and the digestibility co-efficients for *usar* grass are tabulated in Table V along with figures for the other grasses mentioned above.

TABLE V

Percentage composition on dry basis of usar and of some cultivated and indigenous grass hays

Name of grass	Percentage composition			Digestibility co-efficient		
	Crude protein	Ether extract	Total carbohydrates	Crude protein	Ether extract	Total carbohydrates
<i>Usar</i>	6.12	1.02	84.02	42	41	43
<i>Anjan</i>	4.87	0.83	84.12	35	30	59
<i>Dhul</i>	11.10	1.38	74.98	54	27	48
Rhodes	6.36	1.16	83.40	49	38	59
Spear	2.97	0.96	87.07	nil.	36	57

A perusal of data in Table V shows that for a mature grass-hay the digestibility co-efficients of crude protein and ether extracts are fairly high and compare favourably with those of Rhodes grass-hay which is of similar composition. On account of the low digestibility of the carbohydrates of *usar* grass-hay its energy value as represented by total digestible nutrients and starch equivalent is rather low (Table VI).

TABLE VI

Digestible nutrients in usar and in some cultivated and indigenous grass-hays

Name of grass	Per 100 lb. dry matter					Per 100 lb. raw material		
	Crude protein	Ether extract	Total carbohydrates	Total nutrients	Nutritive ratio	Crude protein	Total nutrients	Starch equivalent
<i>Usar</i>	2.57	0.42	36.13	39.65	14.4	2.31	35.69	17.5
<i>Anjan</i>	1.17	0.25	49.63	51.00	30.3	1.05	46.71	29.2
<i>Dhul</i>	6.04	0.38	35.16	43.05	6.1	5.44	38.75	28.7
Rhodes	3.11	0.45	48.91	53.02	16.1	2.80	47.72	26.9
Spear	0.00	0.35	49.91	50.71	..	0.00	45.04	25.5

Calcium, phosphorus and nitrogen balances

Under the dietetic condition of the above digestibility trial, calcium, phosphorus and nitrogen balances of the experimental animals have been determined and the data are given in Table VII.

TABLE VII

Calcium, phosphorus and nitrogen balances per day

Animal number	Intake			Output			Balance gm.
	Usar-hay gm.	Rape-cake gm.	Total gm.	Faeces gm.	Urine gm.	Total gm.	
Calcium							
470	5.02	1.65	6.67	9.21	2.03	11.24	-1.57
465	4.03	1.65	5.68	6.50	2.29	8.79	-3.11
440	4.58	1.65	6.23	7.42	2.65	10.07	-3.84
417	5.05	1.65	6.70	8.99	1.59	10.58	-3.88
374	4.23	1.65	5.88	7.40	2.31	9.71	-3.83
359	4.09	1.65	5.74	6.55	1.63	8.18	-2.44
Average	4.50	1.65	6.15	7.68	2.08	9.76	-3.61
Phosphorus							
470	3.38	2.35	5.73	7.12	0.05	7.17	-1.44
465	2.71	2.35	5.06	5.63	0.03	5.66	-0.60
440	3.08	2.35	5.43	6.39	0.05	6.44	-1.01
417	3.40	2.35	5.75	6.45	0.06	6.51	-0.76
374	2.84	2.35	5.19	5.79	0.04	5.83	-0.64
359	2.75	2.35	5.10	5.09	0.05	5.14	-0.64
Average	3.03	2.35	5.38	6.18	0.05	6.23	-0.85

TABLE VII—*contd.*

Animal number	Intake			Output			Balance gm.
	<i>Usar</i> -hay gm.	Rape cake gm.	Total gm.	Faeces gm.	Urine gm.	Total gm.	
				<i>Nitrogen</i>			
470	22.94	11.58	34.52	17.42	18.66	36.08	-1.56
465	18.42	11.58	30.00	12.32	17.58	29.90	+0.10
440	20.94	11.58	32.52	13.94	19.78	33.72	-1.20
417	23.09	11.58	34.67	15.28	18.72	34.00	+0.67
374	19.31	11.58	30.89	11.82	17.54	29.36	+1.53
359	18.64	11.58	30.22	12.00	16.92	28.92	+1.30
<i>Average</i>	20.56	11.58	32.14	13.80	18.20	32.00	+0.14

It is seen from the balance data that inspite of adequate supplies of calcium and phosphorus, the calcium balance is definitely negative and phosphorus also to smaller extent. Although the average nitrogen balance is positive, two out of six animals showed a negative balance. From the figures of urinary excretion it appears that a large portion of the absorbed nitrogen is simply deaminated and excreted through the kidney. The negative mineral balance and high nitrogenous excretions in urine suggest that under *usar* grass feeding, the physiological mechanism of the utilisation of nutrients is seriously upset. This may be possibly due to the unusually high soda and sulphur content. Further work is in progress.

How far the disturbed metabolism, as revealed by the balance figures, has affected the digestibility of the grass cannot be assessed from the present data. But during the short period of experiment the post absorptive metabolism of the nutrients is unlikely to have any marked influence on apparent digestibility and the figures can be accepted as fair approximations of the digestibility co-efficients.

SUMMARY

The digestibility trial conducted with mature *usar* grass-hay showed that the hay is palatable. The digestibility co-efficients of its crude protein, ether extract and total carbohydrates are 42, 41 and 43 respectively and the nutritive value calculated was found to be 2.57 lb. digestible crude protein, 39.65 lb. total digestible nutrients and 19.4 lb. starch equivalent per 100 lb. of dry hay.

Due to some undetermined factors, the normal metabolism under *usar* grass hay feeding is disturbed, as is evidenced by the negative calcium and phosphorus balance and the large excretion of urinary nitrogen.

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THE COMPOSITION OF SOME INDIAN FISHMEALS

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THE quantitative shortage and qualitative inferiority of stock-feeds in India are well known. To meet these dietetic limitations attempts are being made to augment agricultural productions and also to explore newer sources of feed for cattle. Recently attention has been focussed on the possibility of using fishmeal in the dietary of cattle. In Europe and America fishmeals are fed extensively to swine and in limited quantities to other stock, such as milch cattle, young calves and poultry. The preference for the meal is due to its quantitative richness in protein. Being of animal origin the protein contains the essential amino acids that are lacking in plant products. In contrast to other meals of animal origin, fishmeals contain in balanced proportions large quantities of important mineral matters, such as calcium and phosphorus. It has also been found that the inclusion of a small quantity of fishmeal in an ordinary ration produces a marked increase in the utilization of the feed. There is, however, some prejudice against its use in stock feeding due to the following handicaps:

Whenever the fat content in the meal is above three per cent, it imparts a smell either to the milk or the meat from animals that are fed on it. This smell can, however, be eliminated by reducing the oil content in the product below three per cent and feeding it at a limited proportion in the ration.

Secondly, difficulty, is encountered in the storage of fishmeal. Two factors moisture and fat, play important roles in the storagability of the product. It has been found that the keeping quality can be considerably improved if the moisture content is brought below ten per cent and fat below three per cent.

The present samples represent the average products and were received from the Special Officer, Fish Manure Industry, Calicut. The species from which meals have been obtained are given in Table I. In some cases meals have been prepared from whole fish or from such parts as shells, head, liver, etc. Some of the species have been prepared by more than one standard method.

'Beach drying' and 'cooking and pressing' are the two most favourite methods of manufacture. These methods as indicated by the Department of Fisheries are as follows:

'Beach dried' fishmeal is prepared by spreading the fish on the beach sand individually for drying in the open sun without being cooked and turned over from time to time. Thin fish dries quickly but some sand sticks to the fish in the process of drying. This is then heated to 80°C. and garbled well so as to remove sand. It is then powdered, sieved, fried and stored in tins.

'Cooked and pressed' fishmeal is prepared by heating the fish in a jacketed vessel under gentle heat till the fish is well cooked and steam is developed from the cooking mass. It is then pressed in bags of thick filter cloth in vertical screw presses worked by hand. The press cake is broken into shreads, dried, powdered, sieved, fried and filled while hot in tins.

TABLE I

The species from which meals have been obtained

No.	Name of species	No.	Name of species
1	Blue Mussel (<i>Mytilus viridis</i>)	8	Ribbon fish (<i>Trichiurus sp.</i>)
2	Chank (<i>Turbo inclia pyrum</i>)	9	Sardine (<i>Sardinella fimbriata</i>)
3	Clam (<i>Meretrix sp.</i>)	10	Shark (<i>Carcharias sp.</i>)
4	Mackerel (<i>Rastrellinger kanagurta</i>)	11	Star fish (<i>Pentaceros sp.</i>)
5	Manthal (<i>Cynoglossus semifasciatus</i>)	12	White bait (<i>Stol cphorostoi</i>)
6	Oil sardine (<i>Sardinella longiceps</i>)	13	Unknown
7	Prawn (<i>Penaeus sp.</i>)		

In Tables II and III is given respectively the organic and inorganic composition of fishmeals obtained from the different species. There is a wide variation in the composition. The moisture content varies from 3.51 to 16.00 per cent. As previously indicated the moisture figure in a good quality meal should not exceed ten per cent. Usually the organic matter lies between 77.22 to 86.69 and crude protein between 61.75 to 81.75 per cent. The samples low in organic matter are the ones that are also low in crude protein and vice versa. Star fish is a shell fish and its high ash content accounts for the low figures obtained for organic matter and crude protein. The high insoluble ash or 'silica' in sardines and the high 'silica' combined with excess of sodium chloride in poultry meal made of fish of unknown species account for their low figures for organic matter and crude protein. The desired figure of three per cent for fat is exceeded appreciably in oil sardine but only very slightly in clam, sardine and white bait. The carbohydrates though quite significant in blue mussel and clam are usually negligible in other samples. Meals from mackerel, manthal, oil sardine, ribbon fish, shark and white bait are well balanced with regard to protein, lime and phosphate. In sardines and the meal of unknown species though calcium and phosphorus are well balanced with respect to each other the protein content is rather low for a good quality meal. Moreover in the meal from unknown species the salt content is about 20 per cent though a maximum figure of four per cent is desirable. Although the protein content is quite optimum the value of blue mussel, chank, clam and prawn has suffered due to their low calcium and phosphorus contents. On the other hand, the excessive mineral matter in the star fishmeal considerably jeopardises its merit as a protein feed.

TABLE II

Composition of fishmeals from different species of fish

Organic constituents (per cent on dry basis)

Species of fish	Moisture	Total organic matter	Ether extract	Carbo-hydrate	Crude protein	True protein
Blue mussel	12.54	85.49	1.85	17.89	65.75	60.03
Chank	16.00	86.09	1.36	6.32	79.01	69.30
Clam	9.74	86.12	3.88	16.39	65.85	59.06
Mackerel	12.78	82.04	0.92	5.28	76.74	72.89
Manthal	6.38	77.22	1.14	<i>nil.</i>	80.70	72.09
Oil sardine	8.66	80.64	5.29	5.56	69.79	67.33
Prawn	10.53	90.17	1.78	2.61	85.78	73.11
Ribbon fish	12.63	84.47	0.66	2.06	81.75	79.07
Sardine	5.07	60.92	3.57	0.80	50.55	44.01
Shark	11.51	85.27	1.67	<i>nil.</i>	83.98	73.88
Star fish	3.51	18.83	0.13	10.90	7.80	5.61
White bait	11.98	77.89	3.63	0.53	73.83	67.31
Unknown*	14.28	46.68	1.03	0.43	45.22	32.46

* Meal for poultry

TABLE III

Composition of fishmeals from different species of fish

Inorganic constituents (per cent on dry basis)

Species of fish	Total ash	Insoluble ash	CaO	P ₂ O ₅	MgO	K ₂ O	Na ₂ O	Cl
Blue mussel	14.61	2.58	1.21	1.84	1.55	0.82	3.24	0.94
Chank	13.31	5.63	1.65	1.31	2.40	1.41	0.99	0.04
Clam	13.88	2.22	1.99	2.66	1.63	0.75	2.66	1.63
Mackerel	17.06	1.65	6.17	6.63	1.93	1.52	0.29	0.04
Manthal	22.78	1.31	8.31	8.00	1.12	1.63	0.85	0.98
Oil sardine	19.36	0.82	8.31	7.49	1.05	0.80	0.95	0.04
Prawn	9.83	1.01	1.36	2.46	0.63	1.70	1.34	1.24
Ribbon fish	15.63	0.98	6.49	5.68	1.89	1.63	0.06	0.00
Sardine	39.08	10.82	13.19	10.74	0.71	1.18	0.40	0.53
Shark	14.73	1.74	3.81	3.93	0.99	0.73	1.83	1.49
Star fish	81.17	0.69	40.68	0.16	9.63	1.07	0.96	0.58
White bait	22.11	1.79	9.13	8.64	1.97	1.38	0.40	0.08
Unknown*	53.32	11.07	10.46	7.00	1.94	1.48	11.43	14.19

* Meal for poultry

The meal from the shells of the prawns contains a large amount of ash as compared to the meal from the whole fish. The high ash is also accompanied by high calcium and phosphorus. This increase in ash brings about a reduction in the percentage of organic matter and also of protein. The high protein content of the whole fishmeal makes it a better feeding stuff as the calcium and phosphorus requirements can be met from cheaper sources. There is little difference between the meal made from the whole sardines and that made from the sardine heads. Both the sardine meals however contain oil over three per cent which would preclude their use in the feeding of milch cattle to avoid fishy smell in the milk. Shark head meal is better than the liver meal for reasons more than one. The protein, calcium and phosphorus are considerably higher in the head meal than the liver meal. The latter contains far too much oil to be of any general use in the feeding of animals.

TABLE IV

Composition of fishmeals from different parts of the same fish

Organic constituents (per cent on dry basis)

Description of meal	Moisture	Total organic matter	Ether extract	Carbo-hydrate	Crude protein	True protein
Prawn whole	10.53	90.17	1.78	2.61	75.78	73.11
Prawn-shells	6.15	69.67	0.90	12.83	55.94	42.05
Sardine-whole	5.07	60.92	3.57	6.80	50.55	44.01
Sardine heads	10.41	65.61	3.16	8.88	53.57	45.89
Shark head meal	11.51	85.27	1.67	nil	83.98	73.88
Shark liver meal	8.00	82.76	18.13	28.21	36.42	33.29

TABLE V

Composition of fishmeals from different parts of the same fish

Inorganic constituents (per cent on dry basis)

Description of meal	Total ash	Insoluble ash	CaO	P ₂ O ₅	MgO	K ₂ O	Na ₂ O	Cl
Prawn whole	9.83	1.91	1.36	2.46	0.63	1.70	1.34	1.24
Prawn-shells	30.33	8.20	7.36	4.00	1.25	1.36	2.46	3.04
Sardine-whole	39.08	10.82	13.19	10.74	0.71	1.18	0.40	0.53
Sardine heads	34.39	6.00	13.59	10.87	0.70	0.76	0.76	0.87
Shark head meal	14.73	1.74	3.81	3.93	0.99	0.70	1.83	1.49
Shark liver meal	17.24	1.98	2.53	1.41	1.46	0.71	4.70	4.00

In Tables VI and VII is given the chemical composition of meals as affected by different methods of preparation. In the case of the mackerel samples, except for specific mention in the case of one sample which was marked 'cooked and pressed' the methods of preparation were not supplied to us. In the case of manthal and prawn the method of preparation was clearly stated. When the data of these samples are perused it is seen that the protein content of the samples 'cooked and pressed' is higher than those of 'beach dried'. This apparently large protein content finds explanation from the figure of total ash. 'Beach dried' samples contain a significantly larger quantity of ash. The insoluble ash or 'silica' is also marked high. These observations suggest that 'beach dried' samples in the course of preparation collect considerable amount of extraneous material such as dust and sand. Lime and phosphate content of 'beach dried' manthal is lower than that of the 'cooked and pressed' sample. The prawn samples are rather remarkable in their widely variable disposition in these two minerals. In this case both the constituents of 'beach dried' meal are markedly higher than those of 'cooked and pressed' meal. With the limited information in hand it is difficult to explain this apparent discrepancy. The data in Table VII also suggests that the beach dried samples contain slightly more sodium chloride which may have been picked up externally.

TABLE VI

Composition of fishmeals prepared from the same fish by different methods

Organic constituents (per cent on dry basis)

Description of meal	Moisture	Total organic matter	Ether extract	Carbohydrate	Crude protein	True protein
Mackerel	12.78	82.94	0.92	5.28	76.74	72.87
Mackerel—cooked and pressed	3.66	83.07	3.91	4.60	74.56	67.03
Mackerel meal	7.81	85.48	5.28	6.76	73.14	71.13
Manthal—beach dried	4.96	65.21	2.51	2.01	60.63	53.85
Manthal—cooked and pressed	6.38	77.22	1.14	NH	80.70	72.09
Prawn—beach dried	3.49	69.50	1.17	3.95	64.38	51.81
Prawn—cooked and pressed	10.53	90.17	1.78	2.61	83.78	73.11

TABLE VII

Composition of fishmeals prepared from the same fish by different methods

Inorganic constituents (per cent on dry basis)

Description of meal	Total ash	Insoluble ash	CaO	P ₂ O ₅	MgO	K ₂ O	Na ₂ O	Cl
Mackerel	17.06	1.65	6.17	6.53	1.93	1.52	0.29	0.04
Mackerel—cooked and pressed	16.93	0.75	6.98	6.42	0.53	1.00	0.64	0.38
Mackerel meal	14.52	0.31	6.29	6.12	1.14	0.73	0.40	0.01
Muthal—batch dried	34.79	17.50	6.00	5.73	1.24	1.87	1.34	1.53
Muthal—cooked and pressed	22.78	1.31	8.31	8.00	1.12	1.63	0.85	0.98
Prawn—batch dried	30.50	11.66	6.13	4.37	0.94	1.83	1.53	1.79
Prawn—cooked and pressed	9.83	1.91	1.36	2.46	0.63	1.70	1.34	1.24

CONCLUSION AND SUMMARY

The widely variable composition of fishmeals suggests their suitable blending and grading before they can be used as stock foods. Star fish meal though not a very valuable feed by itself can suitably be used in mixing with other meals like those from blue mussel, clam and clam which have a high protein and low calcium and phosphorus contents. If the blending and grading are carried out successfully and the composition of fishmeal standardized with the products in use in overseas countries, a pound of fishmeal on digestible protein basis would be almost equivalent to two and half pounds of commonly available concentrate mixtures fed to cattle in India.

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INHERITANCE OF SYNDACTYLISM IN HARIANA BREED OF CATTLE

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(With three text-figures and Plate V)

MOST congenital abnormalities found in livestock effect their economic utility. They are, therefore, of considerable importance in animal breeding and production, specially when they have some hereditary basis. Lerner [1944] has listed all the structural abnormalities in Farm animals, which have been accepted as heritable. Hutt [1946] has also pointed out some hereditary structural and physiological abnormalities in domestic animals and has discussed the nature of their inheritance and implication. Atkeson *et al* [1943] reported on bowed pasterns in the hind legs of Jersey cattle. The effected pastern is turned a little outward from its normal alignment, causing the hoof to be placed on the ground with an inward angle, and interfering with quick movement of the animal. The deformity is of a varying nature and, though more readily seen in adults, is undoubtedly congenital. The mode of its inheritance is, however, not certain. Mead *et al* [1943] have reported Flexed Pasterns in Jersey cattle, where the fore-feet were invariably affected, though at times unequally. In severe cases the hoof simply turns back at the pastern and the calf can at best strut about on knuckles. Severity is generally maximum at birth and is normally overcome in about two months time. The defect appears to be of the non-hereditary as well as hereditary-single autosomal recessive type. In Hereford cattle, Morrill [1945] has recorded the appearance of an extra appendage or toe in the fore-feet of the male calves only. As the animals grow, their feet become tender and lame, and the factor has been described as sub-lethal in effect. It appears to be a sex-linked recessive character, only half the male progeny sons of carrier females showing the defect. Reduced phalanges is another defect reported in cattle. In this condition, reported by Johanson in Sweden [Hutt, 1946], the legs are normal but both meta-carpals and meta-tarsals are considerably shortened and the first two phalanges are missing in both digits of all four-feet. The third phalanges and hooves are normal. But as the hooves are attached by only half an inch of skin and tendons, they become useless loose appendages which bend aside when the calves try to stand. As a result, the calves can only crawl about on their knees and hocks. The character is considered as sub-lethal, since the calves cannot survive without special attention. In a study of 13 such creeper calves, it was found that they had a common origin and arose as a result of inbreeding and that a single recessive gene was responsible for the defect. Ross and collaborators [1944] have described the occurrence of syndactylism in the hind-feet of swine due to prenatal malnutrition. Postnatal feeding on minerals and vitamins did not help and the affected animals died.

In India all abnormalities of the foot or leg which affect the locomotivity of the cattle are of special significance as oxen provide by far the largest motive power in the Indian agrarian economy. The subject of the present paper, namely, Syndactylism of the fore-feet in Haryana Breed of Cattle, stationed at Madhurikund Cattle Farm at Muttra (U.P.), was first noticed in 1937 and was reported by one of the authors [S. Singh] in 1942.

In bovines, the normal hoof is bifurcated and has a wider solar surface at the bottom than at the top. The defect reported herein consisted of uncloven hoof but not quite like the equine hoof. It rather resembled an inverted cone with its broad end attached to the pastern and the point and a side in contact with the ground. The surface of foot-and-ground contact was thus comparatively very small, making balanced carriage of the body and its efficient movement difficult. However, only the fore-feet were affected and the animals moved about with a limping gait. Of the 11 abnormal calves observed, two were males and nine females (Table I). With the exception of one female calf, all the other calves showed the defect bilaterally in the fore-feet (Plate V, fig. 1). This female calf, however, showed the defect only in the right fore-feet (Plate V, fig. 2).

TABLE I

Syndactyle Haryana calves born between 1937 and 1941, Madhurikund Cattle Farm, Muttra, U.P.

Serial number	Brand number	Birth date	Sex	Sire	Dam	Remarks
1	983/68(49)	18-6-38	♀	655	689	Only the right fore-foot affected
2	40/49(27)	14-4-39	♂	277	841	Both fore-feet affected
3	N41(31)	20-3-40	♂	373	841	
4	(28)	19-4-39	♂	655	767	
5	135/90(61)	3-9-40	♂	373	680	
6	154/120(80)	27-12-40	♂	655	674	
7	251/21(10)	7-2-41	♂	655	851	
8	205/101(52)	8-10-41	♂	655	812	
9	137/90(63)	5-9-40	♂	373	721	
10	979(23)	5-3-37	♂	277	763	
11	131/70(57)	24-7-40	♂	373	801	

The first abnormal calf was born on 5 March 1937. All of them were found to be otherwise normal and grew up satisfactorily, except that the efficiency of their movement was greatly reduced. Naturally they could not be of any use for working purposes. As no such animal had died on the farm and no post mortem examination result for the defective hoof was available, it could not be said whether the inner vestiges of the toes were in the normal duplicate, or were uncleft. The two rudimentary digits, normally found at the back of the fetlock joint, were present as usual.



FIG. 1. Bilateral syndactylism in Hariana calves



FIG. 2. Unilateral syndactylism in Hariana calves

GENESIS

The possibility of the defect arising from some prenatal malnutrition is ruled out by the fact that only a small fraction (11) of the total progeny of 748 calves, born during the period of incidence of the defect [1937-41], showed the character.

When pedigree (Figs. 1, 2, & 3) of all the 11 defective calves were studied, it became certain that the character had some hereditary basis. It was found that three sires (277, 373 and 655) and nine dams (674, 689, 721, 763, 767, 801, 812, 841 and 851)—all phenotypically normal—were the immediate parents of the abnormal calves and that the sires 277, 373 and 655 and all descended from the same male, viz., grandsire 313. It was further found that in case of eight calves (Fig. 1, i to viii, 313 figured as the common grandsire from both the paternal and maternal lines. It, therefore, became evident that inbreeding had exposed the defect and that the respective sires and dams were normal and carriers of the gene or genes responsible for the expression of the defect.

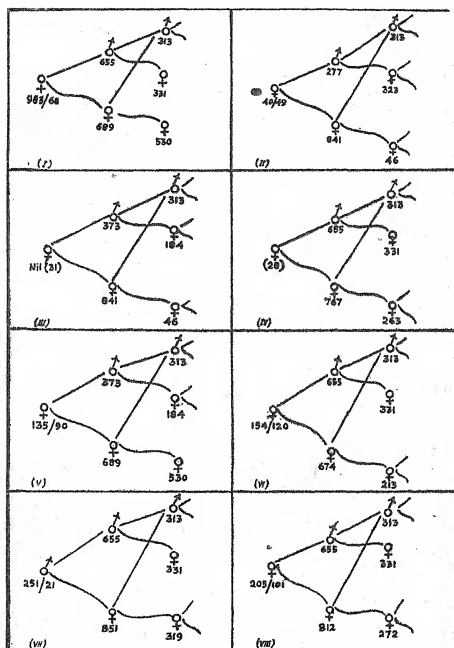


FIG. 1. (Pedigrees I-VIII) Showing how inbreeding has caused the appearance of the character.

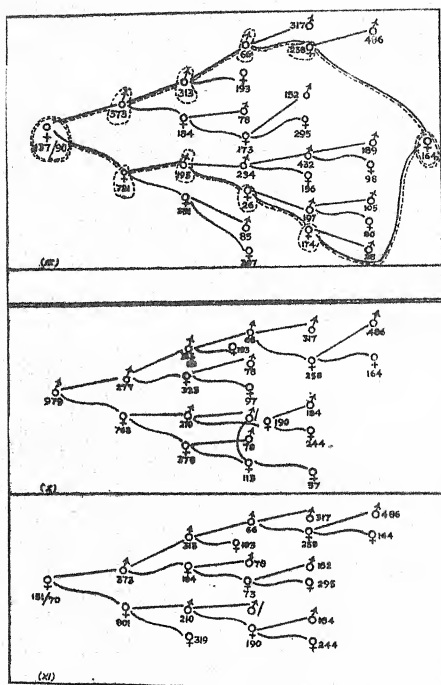


FIG. 2. (Pedigree IX) Traces the common ancestor to 6th generation

FIG. 3. (Pedigrees X-XI) No apparent inbreeding

Pedigree of ♀ 137/90 (Fig. 2) showed a common ancestor (♀ 164) only in the 6th generation. One line of descent was ♀ 258 → ♂ 366 → ♂ 313 → ♂ 373 →, and the other line was ♀ 174 → ♀ 26 → ♂ 195 → 721 → ♀. All these individuals seem to have served as carriers and a convergent breeding of these lines resulted in the abnormal individual combining the full complement of the genes responsible for the expression of the character.

In the remaining two cases, however, i.e., ♂ 979 and ♀ 131/70 (Fig. 3), we have failed to establish any relationship among the ancestors. It is, however, interesting to note that the grandsires 313, 195 and 210 were all brought from Hissar livestock Farm in the years 1927-30. In case of 313 and 195, it has already been established (Fig. 2) that they had a common ancestor in ♀ 164. Assuming that the mutation had its origin in a common ancestor of all these sires, which may be ♀ 164, or some other remote ancestor—which is very likely—it may be further assumed that ♂ 210 is also a carrier like males 313 and 195. This view gets further support from the fact that male 210 appears as the common grandsire from dams' side in both the cases. It seems, therefore, probable that these two defective calves inherited the defective genes from grandsires 313 and 210 by way of their sire and dam respectively.

Mode of inheritance

The foregoing discussion gives clear indication regarding the recessive nature of inheritance of the character. The recessive gene hypothesis receives further support from the following facts:

The possibility of a simple dominant mutation causing the defect is ruled out because in an extended period of five years of incidence, out of a total of 748 young born, there are only 11 abnormal cases. Had there been a dominant gene responsible it would have come to light in a much larger proportion. Also none of the immediate parents or half brothers or sisters showed the character. If the character arose as a new dominant mutant, it would imply that the mutation occurred several times and only in a selected period of five years which does not seem feasible. Furthermore, the fact that the defect was exposed in each case due to established or suspected inbreeding or common ancestry also lends support to the hypothesis that a single dominant mutant is not involved.

A simple sex-linked recessive gene also does not appear to be concerned because we have nine defective daughters, all from unaffected sires. With sex-linked recessive mechanism, a daughter cannot express the defect unless she receives the gene from the X-chromosome of each of her sire and dam.

A simple sex-linked lethal gene also does not appear to be responsible for the inheritance of this defect because that would kill all the affected males, whereas we have got two affected male calves.

It, therefore, seems reasonable to assume that the character is autosomal and recessive. For genetical analysis as to the exact mode of its inheritance, full progeny of the immediate parent-pairs, heterozygous for the defect, was examined (Table III). It was found that out of 17 young born, there were only six normal and eleven abnormal. These figures do not fit in with the Mendelian ratio of 3 : 1 as expected in the progeny from monofactorial heterozygous matings. But perhaps it would be hazardous to come to a definite conclusion with such scanty data. It may, however, be inferred that some autosomal recessive mechanism is involved in producing this defect, though it would be difficult to state at this stage whether it is a simple recessive. There is evidently more in the genetic make up than meets the eye. Study of Tables II, III and IV shows wide discrepancies in the sex ratios of the entire herd, of the Full and Affected-progenies of involved or carrier sires and of the Full and Affected-progenies of carrier parent-pairs. If the numbers

available are to be relied upon, there is a very significant drop in the proportion of males born from Group I to Group V. The data, however, need to be strengthened in this respect before some definite interpretation can be arrived at.

TABLE II

Full progeny of each sire involved

Serial No.	Sire	Calves born			Abnormals			Remarks
		♂	♀	Total	♂	♀	Total	
1	277	1	3	4	1	1	2	} Both fore-feet abnormal 1 abnormal ♀ had only 1 foot abnormal probably right fore-foot
2	373	1	4	5	..	4	4	
3	655	8	10	18	1	4	5	
		10	17	27	2	9	11	

TABLE III

Full progeny of each parent-pair involved

Serial No.	Pair's sire	Involved dam	Calves born			Abnormal			Remarks
			♂	♀	Total	♂	♀	Total	
1	655	689	1	2	3	..	1	1	Only the right fore-foot abnormal
2	277	841	..	1	1	..	1	1	Both fore-feet abnormal
3	373	841	..	1	1	..	1	1	do.
4	655	767	..	1	1	..	1	1	do.
5	373	689	..	1	1	..	1	1	do.
6	655	674	2	2	4	..	1	1	do.
7	655	851	1	..	1	1	..	1	do.
8	655	812	..	2	2	..	1	1	do.
9	373	721	..	1	1	..	1	1	do.
10	277	763	1	..	1	1	..	1	do.
11	373	801	..	1	1	..	1	1	do.
			5	12	17	2	9	11	

TABLE IV

Sex ratios of the entire herd and of the progenies of the involved parents

Group No.	Group	(Sex ratio in males/100 females)
1	Sex ratio for the entire herd for this period of incidence (1937-41)	109.52
2	Sex ratio in the involved sires' full progeny	59.0
3	Sex ratio in the involved sires' affected progeny	22.2
4	Sex ratio in the involved parent-pairs full progeny	41.7
5	Sex ratio in the involved sires' affected progeny	22.2

A further complexity to the problem is added by the fact that, though syndactylism is restricted to only the fore-feet, there is one solitary instance in which it occurred in only the right fore-foot, the left fore-foot and the two hind feet were normal.

A satisfactory answer to the exact mode of inheritance would be difficult unless crosses of the normal and carrier with the affected and between two affected animals are obtained.

SUMMARY

Eleven pedigrees showing both unilateral and bilateral congenital Syndactylism of the forefeet only, in the Haryana breed of Indian cattle have been reported and studied.

All known carriers were normal and the expression of the character came to light as a result of inbreeding.

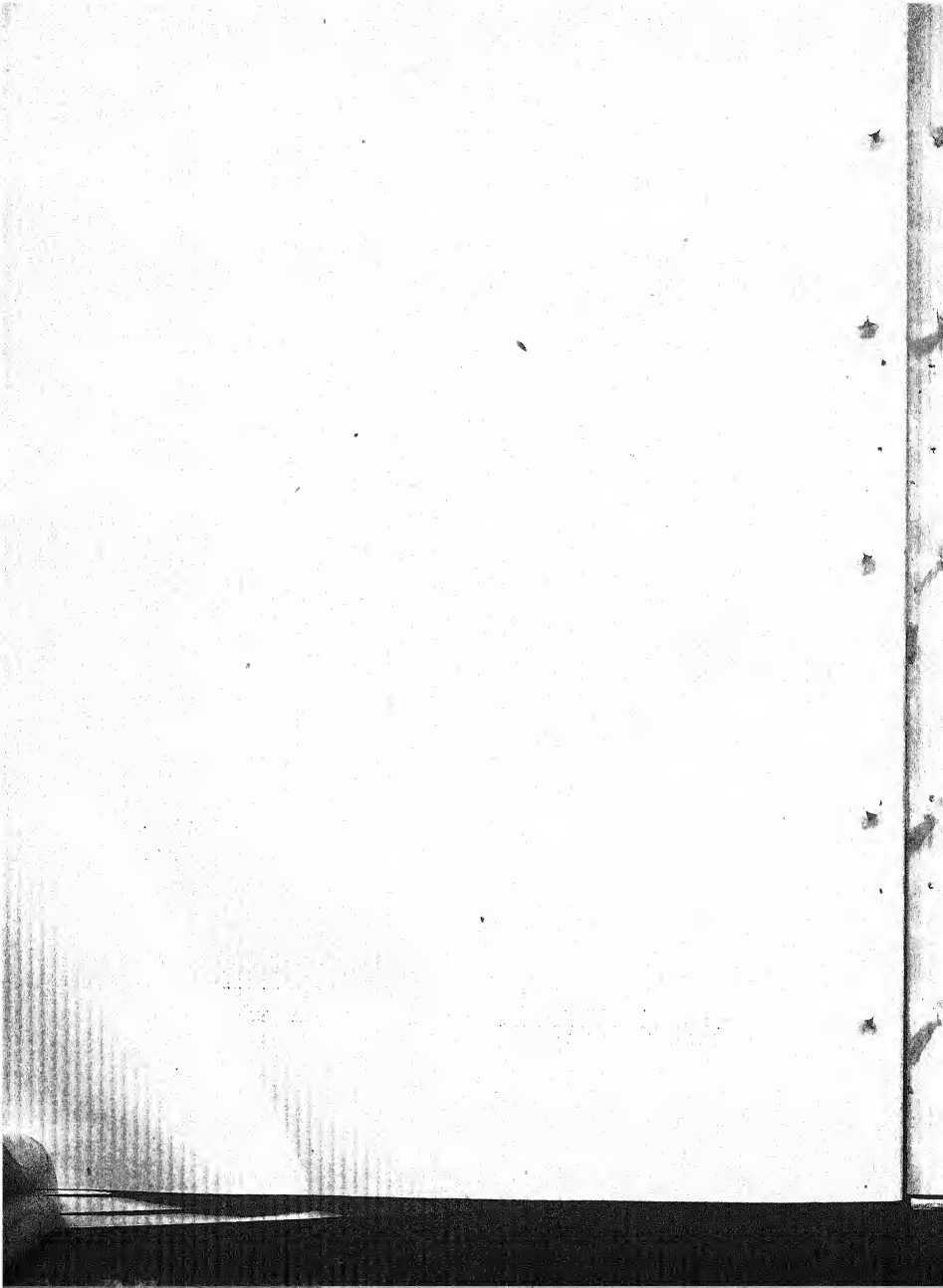
The data available are scanty but are indicative of the operation of an abnormal recessive mode of inheritance.

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STUDIES ON THE SEMEN CHARACTERISTICS OF INDIAN BREEDS OF LIVESTOCK

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WITH the increased use of artificial insemination in livestock breeding, the evaluation of semen quality has gained great importance, particularly where large number of females are being bred to a given sire. Periodical checking of semen quality of sires in continuous service is highly desirable. It enables not only early detection of possible impaired fertility in males due to poor quality semen but also helps in keeping wastage and loss due to a failure in carrying out a pre-planned breeding programme to the minimum. Efficient evaluation of semen quality is essential for numerous laboratory studies, *e.g.*, comparison of semen quality obtained from males of the same breed or species under different dietary or environmental condition or under different hormone treatments, etc. The work of Williams and his collaborators [1920 a, 1920 b, 1925, 1927] focussed attention on the role of the male in impaired fertility of herds, and led to considerable work on the semen of farm animals. The work has been recently reviewed by Anderson [1945].

Great variation is known to exist both in the quantitative and qualitative characters of semen produced by various animals. Even in the same animal, it has been shown [McKenzie and Berliner, 1937; Gunn *et al.*, 1942; Anderson, 1941 a; and 1941 b; Erb, Andrews and Hilton, 1942] that the characteristics of the semen produced at different times also vary. So far no systematic study has been carried out on the semen characteristics of the different species and breeds of Indian farm animals. With the introduction of artificial insemination in India [Guha *et al.*, 1947], the necessity of knowing the normal characteristics of semen produced by the Indian farm animals was realized. The present paper deals with the semen characteristics of the following breeds of Indian livestock:

A. Cattle

- (i) Hariana
- (ii) Sahiwal
- (iii) Kumauni hill type

B. Buffaloes

- (i) Murrah
- (ii) Murrah and local cross

C. Sheep and goats

Types as found in the United Provinces

No single criterion is known so far to represent adequately the true quality of semen [Milovanov, 1934; Walton and Edward, 1938; Davis and Williams, 1939; Anderson, 1939, 1940, 1941a; Hermon and Swanson, 1941]. It is now generally agreed that the most efficient method of semen evaluation is through a consideration of a number of recognized criteria, viz. (i) physical appearance, (ii) volume of the ejaculate, (iii) degree of initial motility of sperms, (iv) sperm concentration, (v) total number of sperms per ejaculate, (vi) percentage of abnormal sperms, (vii) hydrogen ion concentration and (viii) respiratory rate of the sperms. All the above attributes, except the respiratory rate, have been studied and are dealt with in this paper.

MATERIAL AND METHOD

The experimental animals consisted of one Hariana bull, one Sahiwal bull, five Kumauni hill bulls, one Murrah buffalo bull, one Murrah-cross buffalo bull, and five bucks and five rams (of the type ordinarily found in U.P.). Except in the case of Sahiwal bull from which only 18 samples were taken, 24 samples per male were studied in all other cases. All the animals were in good health and in regular service during the period of observation. The semen samples were collected in artificial vagina as described by Walton [1938].

Colour and consistency were determined by the appearance of the semen. Volume was measured up to the nearest 1/100 of c.c. Initial motility of spermatozoa was scored according to the criteria recommended by Erb, Andrews, and Hilton [1942]. Concentration of spermatozoa was determined by Fuch's Rosenthal Haemocytometer and was expressed as number of spermatozoa per c.c. The percentage of abnormal spermatozoa was determined by the method adopted by Mukherjee and Bhattacharya [1947]. pH was determined with the use of a B.D.H. capillator immediately after collection.

RESULTS AND DISCUSSION

The range and mean of the various characteristics studied are brought out separately for individual animals of each breed, and for each of the different species, in Tables I to IV. Table I deals with the bull semen; Table II with that of the buffalo; Table III with that of ram and Table IV with that of goat. In cases where more than one male from a breed were studied, the overall average, range and means are also shown in the tables.

(i) *Physical appearance*

Bulls. The ejaculate of a normal bull of good fertility is opaque white or yellowish white fluid with milky or creamy appearance [Lagerlof, 1934; Kuhne, 1936]. The appearance of the ejaculate depends on the concentration of the spermatozoa, usually watery semen of light yellow colour contains few spermatozoa, although a milky sample may have occasionally few or no spermatozoa [Anderson, 1945]. The physical appearance of the samples observed by us is confirmatory to that observed by foreign workers.

TABLE I
The range and mean of the various characteristics of bull semen

Breed and number of animals	Sperm concentration in millions per c.c. of semen			Volume per ejaculate in c.c.			Total number of spermatozoa per ejaculate in millions			pH			Percentage of abnormal spermatozoa		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Karnaud Bull:															
No. 248	45	1440	460±72	0.37	2.67	1.32±0.41	75	2595	604±110	6	6.6	6.27±0.03	1	28	5.7±1.00
No. 240	64	1560	672±80	0.30	3.60	1.44±0.12	141	3744	958±103	6	6.6	6.18±0.034	1	33	9.1±1.70
No. 195	85	1050	831±80	0.40	4.50	2.01±0.21	260	6412	1383±159	6	7.0	6.21±0.046	0	11	4.3±0.51
No. 194	105	1120	660±37	0.40	4.50	2.37±0.23	96	2927	1682±211	6	6.6	6.14±0.03	1	24	9.0±1.3
No. 187	24	800	322±40	0.02	4.77	2.84±0.19	67	2227	581±113	6	6.4	6.22±0.02	0	11	4.9±0.53
Over all average	23	1250	394±34.8	0.29	4.77	2.00±0.19	67	6412	1192±98.2	6	7.0	6.204±0.018	0	33	9.5±0.54
Haridra:															
No. 12	575	1870	1435±28.3	0.60	6.29	3.10±0.20	600	7812	4344±370	6	6.6	6.18±0.023	6.5	8.9	7.2±0.12
Sahyad:															
No. 11	420	2020	1476±127.0	0.89	9.69	3.80±0.25	1844	16389	5536±308	6.2	6.6	6.25±0.025	7.4	9.4	8.2±0.14

TABLE II
The range and mean of the various characteristics of buffalo-bull semen

Breed and number of animals	Sperm concentration in millions per c.c. of semen			Volume per ejaculate in c.c.			Total number of spermatozoa per ejaculate in millions			pH			Percentage of abnormal spermatozoa		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Marrich:															
No. 1	208	725	609±34.9	0.48	3.6	2.68±0.12	254	2394	1332±8.71	6.2	6.6	6.31±0.028	5.0	9.3	7.2±0.251
Over all average	212	765	692±25.6	0.89	3.8	1.61±0.13	240	1687	841±54.4	6.0	6.4	6.23±0.003	6.2	11.3	8.3±0.257
Over all average	208	765	631±13.6	0.48	3.8	1.84±0.069	240	2394	1036±58.8	6.0	6.6	6.27±0.021	5.0	11.0	7.70±0.15

TABLE III

The range and mean of the various characteristics of ram semen

Breed and number of animals	Sperm concentration in millions per c.c. of semen			Volume per ejaculate in c.c.			Total number of spermatozoa per ejaculate in millions			pH			Percentage of abnormal spermatozoa		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
74	1050	5550	2558 ± 200	0.20	1.20	1.075 ± 0.050	320	3360	1641 ± 212	5.9	6.8	6.28 ± 0.072	0	21	5.17 ± 0.070
46	1100	5550	3160 ± 248	0.25	1.50	0.712 ± 0.066	308	8825	2556 ± 374	5.9	6.7	6.1 ± 0.043	0	13	5.75 ± 0.081
47	1400	7200	3850 ± 280	0.10	1.30	0.073 ± 0.003	574	7020	2009 ± 350	5.9	6.2	5.60 ± 0.01	0	22	4.60 ± 1.143
49	550	8200	3290 ± 242	0.10	0.86	0.55 ± 0.042	172	5825	1019 ± 284	5.9	6.6	6.11 ± 0.008	0	17	4.58 ± 0.082
27	850	6000	3400 ± 229	0.20	0.97	0.666 ± 0.047	496	4158	2273 ± 225	5.9	6.5	6.08 ± 0.043	0	14	5.02 ± 0.078
Over all average	550	8500	3285 ± 128.7	0.1	1.30	0.65 ± 0.025	172	8825	2509 ± 188	5.9	6.8	6.10 ± 0.019	0	22	4.50 ± 0.42

TABLE IV

The range and mean of the various characteristics of goat semen

Breed and number of animals	Sperm concentration in millions per c.c. of semen			Volume per ejaculate in c.c.			Total number of spermatozoa per ejaculate in millions			pH			Percentage of abnormal spermatozoa		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
8	1800	4700	3010 ± 211	0.10	1.20	0.067 ± 0.001	290	4017	1.87 ± 218	5.6	6.6	6.15 ± 0.003	1	7	4.1 ± 0.41
40	650	2800	2507 ± 329	0.12	1.52	0.03 ± 0.005	363	9535	1.90 ± 4.5	6.1	6.7	6.38 ± 0.003	0	10	3.4 ± 0.50
168	850	6050	3000 ± 250	0.14	1.08	0.02 ± 0.003	90	6882	1.02 ± 213	6.0	6.5	6.41 ± 0.009	1	8	3.2 ± 0.46
100	1200	5600	3006 ± 255	0.10	0.78	0.34 ± 0.06	202	2520	1000 ± 148	6.0	6.6	6.38 ± 0.003	1	40	5.4 ± 1.21
109	650	7500	2350 ± 285	0.44	1.70	1.00 ± 0.037	731	11250	2375 ± 449	6.1	6.9	6.40 ± 0.042	0	16	5.3 ± 0.7
Over all average	650	7500	2744 ± 125.8	0.10	1.70	0.65 ± 0.012	90	11250	1500 ± 100.5	5.9	6.9	6.35 ± 0.018	0	40	5.7 ± 0.47

Buffalo bulls. According to Veeramani Ayyar [1944], who studied 16 samples of semen from two buffalo bulls, the appearance is slimy, yellowish milky. In our study, semen samples were of 'thin milky' to 'thick milky' consistency of a whitish colour.

Rams. Ram semen is usually creamy in appearance and as in the case of bulls a thinner consistency and milky or watery appearance has less number of spermatozoa [Anderson, 1945]. In our observations almost all the samples were either of 'thin creamy' or 'thick creamy' appearance.

Goats. Atabek [1936] described the colour of goat semen as that of a 'boiled potato' and it differs markedly from that of the other species. The range of variation in the present study was from very 'thin creamy' to 'thick creamy'. The samples of thinner consistency designated as 'thin creamy' had a light yellow colour, as observed by Atabek. But those of thicker consistency, designated as 'thick creamy' had cream colour.

(ii) Volume

Bulls. The approximate range of bull semen is 0.5 c.c. to 12 c.c. the average volume of the ejaculate being 4 c.c. [Anderson, 1945]. There is also considerable difference between bulls and in the same bull, from time to time. In addition breed to breed differences also exist [Herman and Swanson, 1941]. Thus in the case of Kumauni hill bulls, the volume varied from 0.30 to 1.77 c.c. (mean 2.0 ± 0.1). In the Hariana it was 0.6 to 6.2 c.c. (mean 3.16 ± 0.2) and in Sahiwal 0.8 to 9.0 c.c. (mean 3.8 ± 0.35) (Table I). The mean volume figures from Sahiwal and Hariana in our experiment approximate Anderson's (1941 a) findings. In a study of 195 ejaculates, he obtained a mean volume of 3.53 ± 1.35 c.c. The mean volume in case of Kumauni hill bulls was markedly lower than that of the other breeds. The differences in the volume of the ejaculates may be due to the differences in the body size of the various breeds as has also been observed by Herman and Swanson [1941] and Anderson [1941a].

Buffalo bulls. In 16 semen samples from two buffalo bulls, Veeramani Ayyar [1944] observed a range of 3 to 4.5 c.c.; the most common volume being 3 c.c. The authors have found a range of 0.5 to 3.8 c.c. with a mean of 1.8 ± 0.1 c.c. (Table II).

Rams. According to Moskovits [1934] the average volume of ram semen is 1 c.c. and according to Milovanov [1934] 1 to 2 c.c. Anderson who examined 200 ejaculates from pure bred Merinos gives the average volume as 0.72 ± 0.1 c.c. Phillips *et al* [1943] who studied semen from rams of Hampshire, Shropshire, Southdown and Karakul breeds found considerable breed as well as individual differences. The average volume in Southdown breed in three rams was 0.3, 0.4 and 0.9 c.c. and the average for Hampshire went as high as 1.5 c.c. In the present study, the range of volume was found to be from 0.1 to 1.5 c.c. (mean 0.65 ± 0.03) (Table III).

Goats. Polovcova *et al* [1936] found the average volume of goat semen as 0.7 c.c. Phillips *et al* [1943] who studied the semen of two goats found the average volume varying from 0.7 to 1.5 c.c. according to seasons. In the present study the volume ranged from 0.1 to 1.7 c.c. with the mean volume at 0.7 ± 0.01 c.c. (Table IV). There is considerable individual difference in this species also.

(iii) *Motility score of spermatozoa*

Bulls. In the present study the most common motility scores of spermatozoa of the Kumauni hill, Hariana and Sahiwal bulls were found to be + + + to + + + +, + + + + to + + + + +, and + + + + to + + + + + respectively.

It was not noticed that (1) initial motility varied less than any other semen characteristics, (2) initial sperm motility for normal bulls was fairly high and (3) initial sperm motility varied significantly with different bulls. Our findings are in keeping with those of Herman and Swanson [1941]; Anderson [1940, 1941, 1942]; and Erb, Andrews and Hilton [1942] respectively.

Buffalo bulls. In 16 samples in two buffalo bulls Veeramani Ayyar observed a motility score range of + + + + to + + + + +, the most common being + + + +. The authors found a motility score range of + to + + + + +, the most common being + + + + to + + + +.

Rams. Semen of rams under investigation showed a uniformly high initial motility, i.e., + + + + + as was found by Anderson [1945] and Phillips *et al* [1943]. Terril [1938] however, noted a great variation in the initial motility of ram spermatozoa.

Goats. Average motility of goat semen as studied by Phillips and his collaborators [1943] was very high. In our present study the initial motility ranged from + + to + + + + +, the most common being + + + + +.

(iv) *Sperm concentration*

Bulls. There exists a wide range of variation in sperm concentration of bull semen, the average number being 600 to 1,000 million per c.c. Anderson [1940, 1941a] in several series of observations on fertile bulls found an average of 600 to 900 millions, with a range of 50 to 2,000 millions per c.c. In one series of 171 ejaculates, he got a mean of $648,540,000 \pm 3,430,000$ sperms per c.c. In the present study the minimum (25 millions per c.c.) was observed in Kumauni hill bulls and the maximum (2,020 millions per c.c.) in case of Sahiwal bull. The Hariana and Sahiwal showed much higher sperm concentration than the hill bulls. The respective means of Hariana, Sahiwal and Kumauni hill bulls were $1,455 \pm 82.3$; 1476 ± 127.9 ; and 594 ± 34.8 millions per c.c. (Table I).

Buffalo bulls. The authors have not come across any reference on the study of sperm concentration of buffalo bull semen. They found a range of 208 to 765 millions (mean 631 ± 19.6 millions per c.c.) (Table II).

Rams. Lambert and Mckenzie [1940] have observed a range of 500 to 6,000 millions per c.c. the most common being 1000 millions per c.c. The data for different Western breeds given by different workers are: 2,850 millions [Moskovits, 1934], 2,000 to 4,000 millions, [Milovanov, 1934], and 2290 millions [Terril, 1938]. Our observations differ from the above workers as regards the maximum number of spermatozoa per c.c. This paper records a range of 550 to 8,200 millions per c.c. (mean 3285 ± 128 millions per c.c.) (Table III).

Goats. Polovcova and Fomenko [1936] found the average concentration of goat semen as 4,000 millions per c.c. Phillips *et al* [1943] found the average sperm

concentration of goat semen ranging from 2,063 to 2,997 million per c.c. according to seasons. This paper records a range of 650 to 7,500 millions per c.c. (mean $2,614 \pm 126.8$ millions per c.c.) (Table IV).

(V) *Total number of sperms per ejaculate*

Bulls. The total number of sperms per ejaculate depends on both the volume of the ejaculate and the concentration of spermatozoa. Anderson [1941a] found a range of 616 to 11,000 millions with an average of 5,400 millions per ejaculate. Highly significant differences have been noticed between bulls in the total number of sperms per ejaculate by Erb, Andrews and Hilton [1942].

In this study the total number of sperms per ejaculate in the Kumauni hill bulls ranged from 67 to 6,412 millions (mean $1,192 \pm 98.3$ millions); in Haryana from 600 to 7,812 millions (mean $4,644 \pm 370$ millions) and in Sahiwal from 1,344 to 16,830 millions (mean $5,586 \pm 808$ millions). There was a wide variation among the individuals in the Kumauni hill bulls (Table I).

Buffalo bulls. The present authors observed a range of 240 to 2,304 millions (mean $1,036 \pm 58.8$ millions) (Table II).

There is no other record of work on buffalo bulls.

Rams. The total number of sperms per ejaculate found by different workers varies. According to Moskovits it is 2,850 millions. According to Milovanov 2,000 to 4,000 millions; according to Anderson it is 2,050 millions. Terril [1938] obtained a range of 116 to 7,904 millions with a mean of 2,193 millions. In the present study the range was found to vary from 172 to 8,325 millions (mean $2,260 \pm 138$ millions) (Table III). Individual variation amongst the rams was also noticed.

Goats. Phillips *et al* [1943] found the average total sperms per ejaculate to vary from 2,221 millions to 3,498 millions. In the present study the total number of sperms ranged from 96 to 11,250 millions (mean $1,769 \pm 160.6$ millions) (Table IV).

(vi) *Hydrogen-ion-concentration*

Bulls. Alkalinity of semen is associated with the decrease in activity and concentration of spermatozoa [Anderson, 1945]. This is in conformity with the results obtained in this study. Webster [1932] found a pH range of 7.0 to 7.5; Milovanov [1934] 6.5 to 6.8 with some rare samples showing as low a pH value as 5.5, Anderson [1942] found a mean pH of 6.73 — 0.02.

In the present investigation the respective pH for Kumauni, Haryana and Sahiwal bulls ranged from 6.0 to 7 (mean 6.204 ± 0.018), 6.0 to 6.6 (mean 6.18 ± 0.025) and 6.2 to 6.6 (mean 6.25 ± 0.025) (Table I).

Buffalo bulls. Veeramani Ayyar [1944] from 16 ejaculates of two buffalo bulls has worked a pH range of 5.6 to 6.4. In this study the range was found to lie between 6 to 6.6 (mean 6.27 ± 0.021) (Table II).

Rams. According to McKenzie and Berliner [1937] normal ejaculates with a sperm concentration of 1,000 millions per c.c. are usually acidic; sometimes the pH goes as low as 5.9, and as high as 7.3. Comstock and Brady [1937] gave the

pH of normal semen as 6.9 and of abnormal as over 7.0. Terril [1937] observed that ejaculates giving an acid reaction to litmus, were definitely superior to those giving an alkaline reaction.

The authors are also of opinion that the higher the pH the lower is the concentration. From Table III it can be seen that ram No. 47, with the highest average sperm concentration i.e., $3,850 \pm 289$ millions per c.c., has the lowest mean pH 5.99 ± 0.01 and ram No. 74, with the lowest average sperm concentration of 2538 ± 209 , has the highest mean pH of 6.23 ± 0.072 . But there does not seem to exist much variation in pH among individuals. The pH of ram semen as observed by us, ranges from 5.9 to 6.8 (mean 6.10 ± 0.019) (Table III).

Goats. In this case also it is observed that semen samples slightly acidic in reaction are superior to those which are alkaline. In this study the pH value ranged from 5.9 to 6.9 (with a mean 6.35 ± 0.018) (Table IV).

(vii) *Percentage of abnormal spermatozoa*

Bulls. The following types of abnormal spermatozoa have been found in the present study (1) tailless, (2) headless, (3) bent tails, (4) coiled tails, (5) looped tails, (6) double tails (7) beaded middle piece (8) middle piece very thin and beaded at the neck, (9) pyriform head, (10) micro head, (11) mega-head, (12) double headed (13) degenerated head, (14) deformed head. Most of the above morphological abnormalities have also been observed by Lagerlof [1934] and by Herman and Swanson (1941). The common types of abnormalities encountered by the present authors, were bent tails and tailless.

Williams and Savage [1927] showed that fertility was diminished when abnormal spermatozoa exceeded 17 per cent. Lagerlof believed that when the count of abnormal sperm exceeded 18 to 19 per cent it probably indicated disturbance in the spermatogenesis which may be so serious as to result in impaired fertility. Herman [1940] reported that bulls of good breeding efficiency averaged well below 20 per cent abnormal spermatozoa.

The present authors observed a good deal of variation among individual bulls. Percentage of abnormal spermatozoa for Kumauni hill, Hariana and Sahiwal bulls, ranged from 0 to 33 per cent (mean 6.5 ± 0.54); 6.5 to 8.6 (mean 7.2 ± 0.12) and 7.4 to 9.4 (mean 8.2 ± 0.14), respectively (Table I).

Buffalo bulls. A range of 5 to 11 per cent with a mean of 7.7 ± 0.15 per cent have been found. To the author's knowledge no other work of this type appears to have been conducted on this species.

Rams. All the abnormalities mentioned in the case of bulls have also been observed in the case of rams. McKenzie and Phillips [1934] reported that rams with more than 14 per cent of abnormal spermatozoa are of reduced fertility. McKenzie and Berliner [1937] have recorded as high as 84.8 per cent of abnormal spermatozoa in one ram semen.

In the present study the percentage of abnormal spermatozoa ranged from 0 to 22 with a mean of 4.36 ± 0.42 (Table III).

Goats. Phillips and his collaborators [1943] found the average percentage of abnormal sperms to range from 5.53 to 8.03 according to seasons. The authors noted a range of 0 to 40 per cent with a mean of 4.7 ± 0.047 . There existed a wide variation among the individuals (Table IV).

SUMMARY

Various semen characteristics of Kumauni hill, Sahiwal and Hariana bulls, Murrah and Murrah cross buffalo bulls, rams and goats have been studied.

The means and the average range of variations for each individual as well as for the representatives of the breeds and species considered have been worked out and presented.

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ABSTRACTS

Relationship between Breed of Cattle and Ability to Maintain a Constant Body Temperature under Tropical Conditions., BRIAN, KENDAL. S. (1948). *Vet. J.* 104, 112

LITERATURE quoted by the author reveals that the rectal temperature of cattle of different breeds remain constant in health until the atmospheric temperature reaches 70°F. and between 80-85°F., a pyrexial point is reached and beyond this the animal is not able to maintain heat balance and the temperature goes up very high. It has also been shown that the rectal temperature of an healthy animal will fluctuate with rise and fall of the atmospheric temperature. Large increases in the body temperature may have a deleterious effect on the health of the animal and that a variation in heat tolerance exists between various breeds of cattle.

The author's observations were conducted at Dar-es-Salam in Thanganyika territory to see whether the ability to maintain constant body temperature varied with individuals and between native and imported breeds during cooler and hotter parts of the day. The observations were made on 20 milking cows consisting of six high-grade Kenya Friesians, four low-grade local Friesians, one Zebu European cross-bred and nine Zebus from Kenya. Temperatures were taken at 5-30 a.m. and at 2-30 p.m. in an open sided airy milking shed when at rest. The morning atmospheric temperature during September and October averaged 68-6°F. and at 2-30 p.m. 80-6°F. and in November 70-8 and 86-5°F. respectively.

When the morning, atmospheric temperature was below 70°F. there was no appreciable variation in the body temperature of individual cows in the different breeds. But when the evening atmospheric temperature went above 80°F. the Zebus mean body temperature was 101-9°F. as against 102-6°F. for that of the Friesian and most of the latter had higher rectal temperatures than the Zebu cattle. In November when the atmospheric temperatures were higher some of these were again observed and it was found that the Friesian cows showed much higher body temperatures. Some of these showed a temperature of over 104°F. in November indicating the lack of balance between heat production and elimination.

The European cattle suffered considerable discomfort as they showed increased rate of respiration panting and profuse salivation, where as the Zebu cattle remained well. In the Friesian, the milk yield was also reduced. From the herd history it was observed that cows which showed a diurnal rise of about 0-7°F. during September and October did not thrive well.

It is, therefore, suggested that indiscriminate introduction into tropics of European breeds of cattle or other stocks adapted to cold conditions should be avoided as they will not thrive well.

(Those who are interested in animal husbandry matters in India should study this fact more carefully as the atmospheric temperature in the plains in summer months go beyond 112°F. or more in many parts of the country). (M.K.S.)

On the use of Tissue free Media for the Preparation of Blackquarter Vaccine.

(I) Cysteine Hydrochloride broth, (II) Acid Digest of Liver and Meat.

RAJAGOPALAN, V. R. (1947). *Path. Bact.*, 49 (1 and 2), 39-50.

THE use of chemicals like sodium hydrosulphite, reduced iron, and sulphahydral containing agents like cysteine hydrochloride, glutathione and thio-glycolic acid as reducing agents for growing anaerobes aerobically is well known, but this knowledge has not been put into practical use either in the routine cultivation of anaerobes or in the bulk production of anaerobes vaccine.

The earlier work has been reviewed, and the possibility of manufacture of anaerobe vaccines by using cysteine hydrochloride and acid digest of the liver and meat has been investigated. The observations are set out in detail in two parts:

- (i) the use of cysteine hydrochloride has been shown. Cysteine hydrochloride broth (CHB) containing 0.05 per cent. Cysteine hydrochloride with 0.5 per cent. glucose filled in conical flasks up to the neck, heated at 120°C. for half an hour can be inoculated with *Cl. chauvoei* for the bulk manufacture of blackquarter vaccine. Such a media ensures perfect growth and yields a potent vaccine. The pH favouring the growth of *Cl. chauvoei* is 6.8 or a little more alkaline but not less than 5.8. When CHB is used alone it should be inoculated within twenty four hours of its manufacture and used along with glucose. This period can be extended up to five days when heating the media at 120°C for half an hour before use is necessary. *Cl. chauvoei* toxoid or anaeculture mixed with *Cl. septicum* toxoid or anaeculture confers a higher grade of immunity than *Cl. chauvoei* used alone. Black-quarter vaccine made from CHB and glucose is as economical as the present vaccine in use.
- (ii) The use of acid-digest of liver and meat for the cultivation of *Cl. chauvoei* for the manufacture of blackquarter vaccine is set out in detail. Blackquarter vaccine made from the liver and meat digest media is potent and the growth of *Cl. chauvoei* is in no way inferior to other media. The main points of consideration are, viz., (i) digestion of a mixture of meat and liver in equal proportion yields the best medium, (ii) digestion should proceed for five days if at 56°C., and for five hours if at about 100°C.; (iii) digestion in contact with concentrated acid is better than in dilute acid, (iv) neutralization should proceed slowly so that the medium never becomes alkaline at any stage, (v) the optimum pH is 6.8, (vi) the medium is usable for about 15 days and it should be regenerated by heat before use (K. C. S.)

An outbreak of an Aberrant Type of Rinderpest in Tanagnyika Territory F. LOWE, H. T., WILDE, T. K. H., LEE, R. P. and STUCHBERY, H. M. (1947). *J. Comp. Path.* 75, 175

AN outbreak of a disease resembling rinderpest occurred among cattle in the Tobara District of Tanganyika territory. The outstanding features were the extraordinary mildness of the disease with a low mortality rate (five per cent) and limited tendency to spread in cattle which are ordinarily very highly susceptible to rinderpest.

The symptoms in affected animals varied; but the general picture was that there was rise in temperature lasting for three to nine days after an incubation period of four to seven days. Accompanied by general dullness, nasal discharge, conjunctivitis, erythema of the gums and ulceration on the tongue, gums and lips. Severe diarrhoea was never observed.

Experimentally the disease was transmissible by the inoculation of an extract of lymphatic glands of affected animals and after eight serial passages in susceptible cattle the virus was resorted to full virulence. All the cattle which reacted to the virus were subsequently tested with virulent rinderpest virus and were found immune, thereby establishing that the outbreak was a form of rinderpest. The authors observed that whereas filtered blood after vigorous agitation was infective, filtered blood without agitation failed to infect (C. S.)

Sulfaguanidine and Sulfamethazine in the control of Experimental Avian Coccidiosis caused by *Eimeria tenella*. BARBER CLIFFORD, W. *Poul. Sci.* XXVII (1) 60-65

AS an introduction, B states that in the recent experiments carried out by various workers, there was a wide range in the dosage of the compounds, a variation between the time of medication and the time of exposure, as well as in the virulence of coccidial cultures used, thus making the comparison of different compounds difficult. In this paper, sulfaguanidine and sulfamethazine are compared in their efficacy in the control of avian coccidiosis under identical conditions. Four weeks old chicks reared in battery were used in the experiment. Infection was established by feeding a small amount of wet mash, containing a measured quantity of inoculum. Variation was introduced in the system of medication by giving medicated mash continually and also intermittently for a period of 8 to 9 days. Medicated mash was fed 48, 66, 72, or 96 hours after primary coccidial inoculation.

Sulfamethazine at a 0.25 per cent level was more effective in controlling mortality among *tenella* infected chicks, than 1 per cent sulfaguanidine. Intermittent medication with sulfaguanidine at a 1 per cent level was found to be superior to a continuous six-day feeding at a 0.2 per cent level. *E. tenella* infection could be effectively controlled by 1 per cent sulfaguanidine or 0.25 per cent sulfamethazine if intermittent medication was begun not later than 72 hours after the initial infection. Complete immunity was apparent in the inoculated chicks on re-infection.

A preliminary trial with sulfaquinoxaline forming 0.1 and 0.075 per cent of the mash gave better results than the other sulfonamides tested. (H. K. L.)

Treatment of Experimental and Naturally occurring Fowl Cholera with Sulfamethazine. KISER, J. S.,—PRIER, J., and BOTTORFF, C. A., and GREENE, L. M. (1918). *Poult. Sci.*, XXVII (3) 257-262

SULFAMETHAZINE has been tried in the control of fowl cholera, in artificially infested chicks and in natural field outbreaks. Day old, New Hampshire chicks brooded in separate batteries were used in groups of 70, for each trial. Three tenth c.c. of 1 : 100 dilution of *P. avicida* broth culture—grown for 7 hours at 37°C. and held overnight in the chillroom (4°C.) was used for infection. Sulfamethazine and sodium sulfamethazine were given in mash and water respectively, 18 hours before the infection and trial was continued for 5 days after they were infected. As a result of this treatment, mortality was reduced by 65 to 83 per cent in artificial infection and by 45 to 75 per cent in natural outbreaks as, compared to the non-treated control. No toxic effects were observed. An intermittent schedule of treatment was necessary to effectively control the disease in naturally occurring outbreaks. (H. K. L.).

A study, at High Level Altitude, of Reproduction, Growth, Sexual Maturity and Organ Weights CARL, R. MOOR AND DOROTHY PRICE (1918.) *J. Exp. Zool.* 108, 171-216

INVESTIGATIONS in relation to the influence of high altitude on certain aspects of the physiology of reproduction and weights of various organs were carried out under natural conditions. Four colonies were maintained at 600 ft., 7,500 ft., 9,600 ft. and 14,260 ft. Animals (rats, guinea-pigs, and hamsters) representing a homogeneous strain were fed the same diet at the four different stations; other conditions including temperature, caging, weight and care of the animals were also similar. Drinking water and the amount of light could not be controlled.

Mature animals were found to maintain themselves and carry on effective breeding at various levels of altitude. Animals transported at weaning grew normally and their reproductive performance was unimpaired. Motility and number of spermatozoa did not show any deviation from normal. Fertility of the females was also unimpaired; no cases of resorption of embryos were observed. Litters born were healthy and of normal weight but the rate of growth at the highest altitude was retarded and many eventually died.

It has been concluded that since growth after weaning was not impaired, the faulty growth from birth was an expression of impaired lactation. Weight and functional activity of various endocrine glands closely associated with reproduction and also non-endocrine glands did not reveal any abnormal trend in relation to altitude level.

The findings differed from results of studies on animals exposed to conditions simulating high altitude by means of low pressure chambers or of gaseous mixtures containing reduced pressures of oxygen. The authors are of opinion that optimal conditions in such artificial chambers have yet to be perfected to yield comparable results with natural conditions. (A. R.)

The Genetic Relationship between Mortality from Induced and Spontaneous Lymphomatosis. HEISDORF, ARTHUR J., BREWER, N. R., AND LAMOREUX, W. F. (1947). *Poul. Sci.* 26, 67-73

IT was not possible to differentiate between the lines selected under natural exposure for resistance or susceptibility to neoplasms by inoculating lymphomatous tissue subcutaneously. Nor was there any close relationship between the incidence of lymphomatosis among the inoculated birds of the resistant line and the losses suffered by their sibs reared as controls under natural exposure. Highly significant difference ($X^2=34.4$) was noticed between the losses from lymphomatosis in the resistant and susceptible lines by placing lymphomatous tissue in the crops, eyes and nostrils of baby chicks from the two lines. The study further showed a significant positive correlation ($r = 0.18$) between the mortality from lymphomatosis among the birds under natural exposure and similar losses suffered by their full sibs after 'oral dosage' with lymphomatous tissue. [G. P. S.]

Fertility of Bull semen Diluted at 1 : 400 with and without Sulfanilamide. SALISBURY, G. W., AND BRATTON, P. W. (1948). *J. Dairy Sci.* 31, 817-822

FIELD results of two series of experiments involving 356 + 734 insemination using dilution rates ranging from 1 part of semen to 100 to 800 parts of two differently treated dilutors, are discussed. The dilutor tried was the egg-yolk citrate mixture. The treatment consisted in addition or non-addition of 300 mg. of sulfanilamide per 100 ml. of dilutor. In the second series the dilutor was treated with sulfanilamide.

For the first series, a 4×4 Latin square design was used. Each of the 4 ejaculates, all coming from a single, consistently high fertile bull, was split into 4 aliquots with each aliquot diluted at rates of one part of semen to 100, 200, 400 and 800 parts of egg-yolk citrate dilutor and containing on an average 14,580,000; 7,330,000; 3,670,000 and 1,840,000 spermatozoa per ml. of diluted semen, respectively. In all 356 cows were inseminated in this series.

Semen used in the second series came from 19 different bulls. A randomized block design was employed. The 5 dilution rates studied namely 1 part of semen to 100, 150, 200, 300 and 400 parts of dilutor and containing on an average 12,060,000, 8,490,000; 6,340,000; 4,160,000 and 3,290,000 spermatozoa per 1 ml. of diluted semen respectively, were assigned at random to the 5 collections taken from each of the 19 bulls. In this series 7,343 cows were inseminated.

Analysis showed that in the sulfanilamide treated series, though a trend downwards amounting to 0.8 percent in fertility level for each decrease of 1 million spermatozoa inseminated was observed over the range of 2.36 to 15.30 millions of spermatozoa inseminated, none of the differences were statistically significant. Significant differences were, however, observed in the other series, between 1 : 100 dilution rate on the one hand and 1 : 400 and 1 : 800 rates on the other.

The probable reasons for the different results of the two experiments are discussed. The authors suggest that the minimum number of spermatozoa consistent with optimum fertility rests at 5 to 10 millions from bulls of known fertility. (S. S. P.)

condition. Under aerobic condition, oxygen utilisation is of relatively short duration. It can, however, be prolonged by the addition of fructose. Similarly in anaerobic condition, addition of fructose prolongs survival. These findings show conclusively that metabolism of fructose, i.e., fructolysis and not respiration which supplies vital energy to the spermatozoa while in the genital tract or *in vitro* storage. Aerobically, although most tissues can utilise both glucose and fructose, in anaerobic condition, excepting spermatozoa, none of these tissues, including also seminal vesicles where fructose originates, are capable of utilising fructose. (A. R.)

REVIEW

Veterinary Protozoology

By ULICK F. RICHARDSON, B.Sc., M.R.C.U.S. (Published by OLIVER and BOYD, Ltd., Edinburgh, 1948; Price 18s. pp. 240 with 34 illustrations, one coloured plate and numerous references.

THIS book is a first attempt in presenting information on protozoa of veterinary importance in a consolidated form. This will greatly overcome the difficulties of the students as well as the field workers who have to dig up various books to complete their information on the subject. The book is written in a clear and lucid style, which it is a pleasure to read, and includes a very useful list of latest references. The author has not restricted himself to the description of morphological details and life histories of protozoa but has included full details of the methods of control, diagnosis and the symptoms of various protozoan diseases. In reference to various *Theileria* species it is stated that 'the specificity as regards the invertebrate hosts argues that the species are distinct but an open mind should be kept on their relationship to each other, and the possible influence of extraneous factors on their pathogenicity which is the principle characteristics in which they differ from each other' which clearly goes to show that the book is free from dogmatic statements. A general statement, however, that a cutaneous leishmaniasis of cattle occurs in India, should have been qualified, because apparently, it is based on a single case, reported by Pande [1941] in the *Indian Journal of Veterinary Science and Animal Husbandry*.

In addition, the book includes two useful chapters, one on Chemotherapy mentioning the latest drugs and the other on technique, which describes in detail the methods of staining, culture, and preservation of material. (H. K. L.)

Pregnancy Diagnosis Tests—A Review

By A. T. COWIE, B. Sc., M. R.C. V. S., PH. D. (NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING, UNIVERSITY OF READING, 1948; pp. 283—containing 100 pages of bibliography. C. A. B. Jt. Pub. No. 13)

THE author has taken great pains to collect such extensive literature on the subject, as is available in various parts of the world, and presented it in the form of a book. The subject matter has been well classified and is described in ten chapters. The book has attained its object of 'providing the scientific worker with a brief summary of the more important researches leading to the development of pregnancy tests in the domestic animals, and to provide an adequate bibliography of the widely scattered literature'. As result of the survey of this literature, it is concluded, that the pregnancy may be diagnosed in mare in the second month by an expert clinician as well as by laboratory tests, in the cow from the third month onwards, in ewe and goat during the last two months by clinical methods. In dogs and cat it may be possible to diagnose pregnancy from the third week by clinical methods; radiography in this case is of no use till the seventh or eighth week while hormonal tests are of no value. In sow no reliable methods are available, except that the *Oestrogen* test carried out between the 21st and 30th days after service, may be helpful. (H.K.L.)

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Indian Journal of Veterinary Science and Animal Husbandry Vol. XVIII, Part II, June 1948

<i>Page</i>	<i>Line</i>	<i>For</i>	<i>Read</i>
81	9	maize	Maize
81	Table I	Total Calcium ash	Total ash : calcium
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82	3 under experiment I	Nitrogen calcium	nitrogen, calcium
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84	6 under bullock No. 4	Total, digested	Total digested
85	Table V	Nitrogen balance	nitrogen balance
85	10th from bottom	salt and with wheat	salt and wheat
89	16 from bottom	Salubrious	salubrious
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ORIGINAL ARTICLES

STUDIES ON CONTAGIOUS PLEUROPNEUMONIA OF THE GOAT IN INDIA

By J. F. SHIRLAW, Lately of the Indian Veterinary Research Institute, Izatnagar

(Received for publication on 15 January 1949)

ACCORDING to Curasson [1936] the earliest record of the existence of contagious pleuropneumonia of the goat in India was made in 1889 by *Steel who investigated the disease in Khandesh, Bombay Province and later, in 1914, *Walker encountered the same disease in goats in the Punjab. In the archives of the Indian Veterinary Research Institute are available several unpublished references on the disease. Thus, *Oliver [1916] reported the disease as the most serious epizootic of goats in the United Provinces, sheep being unaffected; annual losses varied but were always serious, particularly in hilly areas. The highest incidence occurred in the cold season, and after the first month or two when the peak was reached, the disease smouldered in affected areas until the onset of the hot season. The monsoon ushered in fresh outbreaks, specially in hill grazings. He considered that the disease had much in common with contagious bovine pleuropneumonia. *Taylor [1920] commented on the prevalence of a similar fatal disease of goats throughout the Bombay Province, and considered that the disease was related to contagious bovine pleuropneumonia as similar methods of vaccination were practised in goats by the shepherds, with what results he could not say. In this connection, a leading Bombay hide and skin merchant reported [1919] 80 per cent of losses in goats due to contagious pleuropneumonia. Sheep were not affected and up to 70 per cent of goats could be satisfactorily immunized by the simple expedient of inserting a small fragment of fresh pneumonic lung into a skin incision in the ear. *Vacha [1923] in reporting the prevalence of this disease in Baluchistan makes mention of a similar method of protection practised by herdsmen. Gareval [1923] refers to contagious pleuropneumonia of goats in the North West Frontier Province. Ceylon was invaded by the disease in 1924, goats from Karachi being held responsible for its introduction. *Jerrom [1929] reported a high incidence of epizootic pleuropneumonia of goats in the Sind Province and Rajputana, with sixty-four outbreaks between April 1925 and October 1927 and mortality 70 to 100 per cent. Vaccination practised by herdsmen in Sind certainly gave some protection against the ravages of the disease, the method being to rub fresh pleural exudate into a skin incision on the tail.

In none of these reports is there any accurate description of the disease. The incubation period is stated to be two to fifteen days and the duration of the disease two to twelve days, although some animals may survive for longer periods, even two to three weeks, whilst animals that recover are immune to further attack; generally,

* The references marked with an asterisk are either from unpublished records or correspondence in the archives of the Institute, personal communications or references quoted by Curasson but not traceable to the original.

the disease is acute. The pulmonary lesions include pleural adhesions, hepatization and fibrinous exudate in varying amounts in the chest cavity and pericardial sac. *Sheather [1916 to 1919] considered, after some investigation, that the disease was probably caused by a filtrable virus. He appears to have isolated at least one virus, but no records of these results are available for reference. *Edwards [1923 to 1926] studied strains of an apparent *Pasteurella* organism and organisms of the genus *Corynebacterium* isolated from outbreaks of the disease in the Kumaun Hills. He concluded that these organisms were merely secondary invaders and suggested that the underlying cause of the disease was 'a filterable virus akin to that causing contagious bovine pleuropneumonia'. Records of this work are incomplete but that the conclusion is more than hypothetical is indicated by his later recommendation to attempt vaccination against the disease in goats along the lines successfully practised in the control of contagious bovine pleuropneumonia. However, no steps in this direction were apparently undertaken. Cooper [1927 to 1929] considered a *Pasteurella* organism to be the cause of epizootic pneumonia of goats in the Kumaun Hills; although *Pasteurella*-like organisms were almost invariably found on bacteriological examination of the lungs, their aetiological relationship with the disease could not be established. Since 1933, when Veterinary Investigation Officers were appointed to each province, epizootic pleuropneumonia of the goat had engaged attention in all provinces, and although no accurate surveys of the incidence and losses incurred over a number of years had been made, losses were said to be severe when outbreaks of the disease occurred. In 1944, fifty thousand goats were said to have died of pleuropneumonia in Rajputana; the statement is credible in view of the known large concentrations of goats in parts of India and the devastation that the disease can cause. This was certainly a moderate figure compared with losses due to this disease among goats held by the army during the last few years.

Credit is finally due to Longley [1940] for placing on record a precise account of epizootic pleuropneumonia of the goat as it occurs in Madras Province, and for describing the aetiological factor as a member of the pleuropneumonia group of organisms for which he proposed the name *Barrelomyces peripneumoniae capri*. However, this worker was unable to isolate the organism in culture. He appears to have based his decision on the aetiology on three considerations.

- (a) Specific lesions develop within the muscles and subcutis following the intramuscular inoculation in susceptible goats of a few drops of pleural exudate obtained from cases of pleuropneumonia in goats destroyed at the height of the disease. These lesions are pathologically comparable with those seen in cattle inoculated subcutaneously with virulent cultures of the bovine pleuropneumonia organism.
- (b) Filtrates of pleural exudate produced typical muscle lesions when inoculated subcutaneously or intramuscularly in susceptible goats, and the syndrome of the disease with typical lesions when insufflated in similar animals.
- (c) Growth phases of an organism morphologically resembling the bovine pleuropneumonia organism were recognized on 'dark ground' examination of fresh pleural exudate.

A strictly comparable disease of goats is known to exist in France, Switzerland, Germany, Italy, Anatolia, Greece, Macedonia, Bulgaria, Syria and Palestine. Curasson [1936] has extensively reviewed the literature on the subject and the following facts about the disease in other countries are gleaned from this review.

On the continent of Africa, the disease reaches serious proportions and has a wide distribution. In Algeria and Morocco, it has been known for centuries as a veritable scourge of goats and the name *bou frida* has been coined by the natives to describe the cardinal lesion of unilateral pneumonia. The disease has spread through French West Africa, and the Belgian Congo, and has been extensively studied in Nigeria, Kenya, Uganda and Tanganyika; Egypt, the Sudan, Eritrea and Italian Somaliland are likewise affected. The disease was accidentally imported to South Africa in 1880, and during that and the following year, more than eighteen thousand goats were lost. The disease was stamped out of this country by a rigorous slaughter policy and had not since reappeared. Losses vary in different outbreaks, in the countries listed, but are generally given as 50 to 60 per cent, although this figure may soar to 100 per cent in some outbreaks. In Greece, *Stylianopoulos [1932] averaged the case mortality at 98.5 per cent; of seven hundred and fifty-five goats belonging to different herds only eleven survived whilst in another outbreak involving five hundred and eighty-five goats only nine survived. *Mori [1916] gave the mortality in Italy as about 90 per cent. *Henderson [1928] witnessed a mortality of 100 per cent in Nigeria. The highest incidence and mortality occur during the winter months and the disease smoulders with occasional exacerbations during the summer months. Sheep are quite refractory to the disease in the territories noted. *Metnam [1929] considers this disease to be the most serious of all maladies to which the goat is subject. The lesions of the disease, as given by *Mori [1916], *Stylianopoulos [1933], *Cadenc [1902], *Leclainche [1896], *Thomas [1893], *Metnam [1929] and other workers are as follows: the lung and pleura are exclusively affected, usually on one side. When both sides are affected it is the direct result of a rather prolonged attack of the disease, but, according to Mori, an early bilateral pneumonia can occur. In both peracute and acute forms all phases of a pure fibrinous pneumonia with red and grey hepatization are seen. Hepatization affects all or part of the middle-lobe of the lung, in vertical wedges, the anterior and posterior lobes of the lung remaining unaffected; or, there may be several closely adjoining patches of bronchopneumonia. The affected areas project above the lung surface and vary in shades of red to grey. The appearance of the cut surface is similar to that seen in the lung affected with contagious bovine pleuropneumonia; it is marbled and forms a mosaic pattern as the result of variegation of the lobules which are deep red, bluish, wine coloured or greyish and sharply demarcated by the interlobular connective tissue which is prominent and distended by exudate. Sometimes this infiltration is absent. Thrombi are seen in the blood vessels and the bronchi contain a clear exudate which clots immediately on death. Lesions of pleurisy are rarely absent. In general, the pneumonic areas of the lung are covered with a yellowish layer up to an inch in thickness and saturated with a clear exudate percolating widely through the channels formed by the spongy and soft membrane. The degree of adhesion of the membrane to the lung and to the pericardial sac is variable; in peracute cases, it is easily removed. If the development of the lesions has been slower, it is more

or less adherent to the lung according to the degree of organization. If pneumonia is bilateral, the membranes may be present on the other side as well, but not invariably; quite frequently, there is simply a thickening of the visceral pleura. The exudate in the chest cavity is light yellow in colour, contains no blood, is incolorous and varies in quantity from one half to two litres. The bronchial lymph glands are swollen, infiltrated and haemorrhagic in some cases. The pericardial sac usually contains exudate, sometimes in considerable quantity. *Mettam found bilateral pneumonia in 36.5 per cent of cases, and in the remainder, the right lung was more frequently affected than the left 40.5 per cent as against 23 per cent. *Stylianopoulou stated that, as a rule, the right lung was chiefly affected, and much less commonly both lungs; exceptionally, the left lung alone was affected. Mettam noted neither necrosis nor suppuration. Also there is a general consensus of opinion that sequestra do not occur in this disease. *Leclainche early incriminated a *Pasteurella* as the direct cause, but later modified this finding by distinguishing between *Pasteurella* pneumonia of the goat and enzootic pneumonia which, he considered, had much in common with contagious pleuropneumonia of cattle. *Melanidi and Stylianopoulou [1928] were able to isolate no potentially pathogenic organisms from the lungs and pleural exudate from typical cases of the disease examined immediately after death or destroyed *in extremis*; on the other hand, they isolated *Pasteurella* organism from animals examined some hours after death and concluded that that was a secondary invader. *Mettam [1929] showed that the disease could be reproduced in susceptible goats with filtrates of pleural exudate, an observation which was confirmed by *Walker [1932]. *Kearney [1928] found, after some days, a suspicious opalescence in tubes of Martin's broth sown with heart blood, spleen and pleural exudate from typical cases of the disease. He considered that this growth was identical with that of the bovine pleuropneumonia organism, grown under the same conditions. *Kearney observed a typical thermal reaction and death in five to six days in goats subcutaneously inoculated with pleural exudate or lung mash; a huge inflammatory oedema developed at the site of inoculation, the lesion later becoming hard and fibrous. *Mettam was able, on several occasions, to transmit the disease in its typical form by insufflating healthy goats with pleural exudate, combining this technique with intrapleural inoculation of the same material. *Stylianopoulou transmitted the disease in goats in series by inoculating lung mash from diseased goats directly into the lung, the inoculum in all cases being proved bacteriologically sterile; further, when healthy goats were mixed with experimentally produced cases of the disease, they contracted the typical disease. *Mettam showed that the disease could be transmitted to about 25 per cent of animals experimentally exposed to infection, and *Stylianopoulou stated that an outbreak of the disease in a herd always coincided with the introduction of an animal already infected or in the incubative stage of the disease. This same worker found that the virus maintains its virulence in lung juice or pleural exudate for not less than fifteen days at laboratory and refrigerator temperatures, when stored fresh or preserved with 50 per cent glycerine. Quite a few of those early workers on the disease appear to have achieved considerable success in prophylaxis. Thus I *Hutcheon [1889], working in Cape Province, believed the disease to be analogous to contagious bovine pleuropneumonia and obtained encouraging results on the vaccination of healthy goats with pleural exudate from diseased goats given subcutaneously in the tail.

*Mori [1918] used a vaccine which contained the virus in a viable state, and obtained by allowing the pleural exudate to clot, the supernatant fluid constituting the vaccine. *Crimi [1926] used this vaccine in 2967 goats and concluded that even in badly infected herds, the method was uniformly successful. *Henderson [1929] vaccinated goats with a fresh lung emulsion given subcutaneously and obtained encouraging results; vaccinated animals stood up to the disease whereas 61 per cent of unvaccinated animals contracted the disease and died. *Schellhase [1921] obtained equally encouraging results with the same method. *Brakopoulos (quoted by Sylianopoulo) was successful in protecting animals with a mixture of lung pulp and pleural exudate. *Sylianopoulo claimed good results for a formalized vaccine prepared from a mixture of lung pulp and, pleural exudate.

The purpose of this article is to describe further work on the disease in India, in extension of that originally recorded by Longley [1940].

SCOPE OF THE INVESTIGATION

During the last decade, there has been no dearth of material for investigation into contagious pleuropneumonia of the goat. The disease has been given prominence in the annual reports of nearly all the Provincial Veterinary Investigation Officers. The investigation now recorded was set up in 1942, and as a matter of convenience, outbreaks of the disease which occurred in Bombay Province, during the winter months of 1942, were selected as the main field of investigation. The main objectives were to determine (a) the incidence and mortality in the field, (b) the nature of the disease with reference to its epizootology and pathology, (c) the causal agent, which having been decided, (d) control by vaccination or other suitable measures, viz. (e) therapeutic. It has been claimed by several Veterinary Officers in India and Ceylon that novarsenobillon is an effective remedy in acute cases. It was necessary, therefore, to investigate this claim. The general field investigation was coordinated with work in collaboration with Provincial Veterinary Investigation Officers, chiefly as regards the epizootology, pathology and diagnosis of contagious pleuropneumonia of the goat as it occurred in other provinces. No assumption was made that all outbreaks of contagious pleuropneumonia in India were aetiologicaly identical. Indeed, it is fairly well established that some outbreaks of pneumonia of the goat in India may be related to infection with *Corynebacterium* or helminths, or even with a virus at present under study. It was at once apparent, therefore, that a means of field diagnosis of the prevalent epizootic form of the disease was urgently necessary if any accurate recording of the incidence in the provinces was to be possible. Fortunately, this means of diagnosis became available at an early stage of the investigation. The entire experimental work relating to the isolation of the causal organism and immunity was staged at Izatnagar in locally purchased goats. The work on immunity explored the claims [Longley, 1940] of useful protection by means of formalized vaccines prepared from lung mash or pleural exudate obtained from a natural case of the disease, and also by fresh exudate given subcutaneously either by ear or tail tip. The work finally progressed on the lines of vaccination successfully employed in the control of contagious bovine pleuropneumonia.

Investigation in Bombay Province

Investigation centred in the Dharwar tract. Although the goat population is considerable in this area, it is divided among individuals in small numbers, and the opportunity did not occur of observing the disease in large self-contained herds. Cases of the disease were sporadic and owners stated that that was a disease hitherto unknown in the tract which had been affected for about a month. The animal population in one area was 709 goats, 781 sheep and 759 bovines, and the disease was confined to goats. The losses given by owners were, 5 out of 10, 10 out of 16, 4 out of 10, 44 out of 100 and 40 out of 120. In one particular instance involving 25 goats, 18 were affected with the disease of which 3 survived; such recoveries were, however, exceptional. In another area there were 600 goats, 1,000 sheep and 1,000 bovines, the disease being again confined to goats. The outbreak had commenced two weeks previously and 6 goats, had died, and one recovered. The duration of the disease was given as three to eight days. The symptoms were nasal discharge, at first mucoid, later purulent, cough, temperature up to 106.5°F., staring coat, hurried respiration and standing with arched back, extended neck and the forelimbs set wide apart. Severe terminal diarrhoea ushered in prostration, coma and death.

Pathology: Field cases at Dharwar

Ten cases were examined. The first goat showed extensive consolidation of the middle lobe of the right lung, with a small pleuritic patch over the dorsal surface of the lobe, and no exudate in the chest cavity; the bronchomediastinal glands were enlarged and oedematous. The trachea and the bronchi showed catarrhal change, and the pharynx and larynx some decided congestion. Rhinitis with nasal discharge was present, with no extension into the sinuses which were free of parasites. The second goat showed a patch of pleurisy over the middle lobe of the right lung, but the lung was entirely free of pneumonic change. The lesions of the upper respiratory tract were common to this goat and to those in the series examined. The third goat showed extensive pleurisy on both sides with adhesions to the chest wall, and empyema was marked. The left lung alone was affected, all lobes consolidated, and abscess prominent; examination of the pus showed *G. pyogenes*. The fourth goat showed extensive consolidation of the entire left lung with generalized pleurisy of that side and firm adhesions to the chest wall and pericardium which was considerably thickened; the right side of the chest was unaffected. In the fifth goat, the diaphragmatic lobe of the right lung was consolidated, with a small patch of pleurisy on the dorsal surface. This patch was organized and firmly adherent to the upper rib surface. Pericarditis was well marked, the pericardial sac containing fibrinous exudate. The mediastinal glands were enlarged and more oedematous than in other goats of the series. The left side of the chest cavity was unaffected. In the sixth goat, the right diaphragmatic lobe was alone involved and firmly adherent to the chest wall. In the seventh goat, both lungs were firmly attached throughout all lobes to the chest wall, and covered by a thick layer of partially organized fibrinous exudate; empyema was present on both sides. The left lung throughout showed advanced septic pneumonia with multiple abscess and cavitation. The right side of the lung was normal, only the pleura being involved. Extensive fibrinous

pericarditis was seen with adhesions to the anterior lobes of both lungs and to the floor of the sternum. In the eighth goat, all lobes of the left lung were consolidated and covered with a layer of fibrinous exudate undergoing organization with adhesions to the chest wall and pericardium. No lesions were seen in the right side. The ninth goat showed similar lesions to the foregoing, the right middle and diaphragmatic lobes being involved. The tenth goat, the original donor animal used in the isolation of the 'virus', showed extensive puritic adhesions on both sides, as well as pericarditis. Lesions of pneumonia were restricted to the middle lobes of the right and left lungs. The pleural sac contained about 100 c. c. amber coloured exudate clotting rapidly on removal. Marbling of the lungs was seen in only three goats of the series. To sum up, the essential lesions of the disease seen in field cases were :

- (a) Catarrhal inflammation of the upper respiratory tract, with rhinitis and nasal discharge. As no larvae of *Oestrus ovis* were seen in any of these cases it was assumed that the latter lesion was a specific feature of the disease.
- (b) The cardinal lesions affect the pleurae and lungs. There was a strong tendency for pneumonia to be unilateral, with no predilection either for the right or left lung. Pleurisy, often of a gross degree, might have existed independently and the lung on that side be unaffected ; or, the area of pleurisy might have been almost negligible in comparison with the extent of lung involved in the pneumonic process. The presence of exudate within the chest cavity was by no means a constant feature.
- (c) Consolidation of the lung affected either the middle or diaphragmatic lobe, the anterior lobe escaping involvement. The type of pneumonia was essentially fibrinous and non-septic. However, the original pneumonic process might have been complicated in few cases by a secondary septic infection.
- (d) Pericarditis was a frequent finding.
- (e) There was a total absence of lesions in the abdominal viscera, with the exception of parasitic gastro-enteritis which was a constant finding, and considered to be the origin of the severe diarrhoea seen during the course of the disease.

Experimental transmission of the disease in healthy goats was attempted with materials from four of these cases. The goats used were obtained from a disease-free area about 20 miles away from the affected area. Three goats were insufflated and subcutaneously inoculated in the neck with mixed saline extract of pneumonic lung and pleural exudate from one case. All three goats developed, within three days, a painful inflammatory oedema at the site of subcutaneous inoculation. In two animals, an abscess formed which ruptured, and in the third animal the swelling absorbed within a week. There was no thermal reaction. One goat died 16 days p. i. and showed pneumonia chiefly affecting the left lung with copious fibrinous exudate in the chest cavity, those lesions being similar to those seen in natural cases of the disease. The other two goats remained healthy ; one was destroyed 30 days p. i. and no lung lesions seen. Two more goats were inoculated

subcutaneously in the neck and intratracheally with saline extract of pneumonia lung of the positive reactor. One goat developed a large inflammatory oedema at the site of subcutaneous inoculation extending into the underlying muscles; pneumonia was apparent at the third day and death occurred five days p. i. The lesions seen were unilateral pneumonia, the anterior lobe of the right lung being affected with fibrinous exudate in the chest cavity. The second goat did not react and showed no lesions when destroyed 17 days p. i. One more goat was subcutaneously inoculated and insufflated with saline extract of pneumonic lung from the positive reactor. Nasal discharge and intense local lesion were seen three days, signs of pneumonia six days and death occurred twelve days p. i. with lesions of pleurisy, but no pneumonia. There field cases were examined bacteriologically. No organisms were seen in films from heart blood or pneumonic lung stained by Gram, Loeffler or Giemsa, and broth and blood agar tubes sown with these materials were sterile after three days incubation at 37.5°C. Rabbits subcutaneously inoculated with saline extract of diseased lung from the three cases did not react which appeared to negative a possible *Pasteurella* infection.

Transmission experiments at Izalagar

The seed material derived from a clinical case sent from Dharwar. On autopsy, the chest cavity contained about four litres of fibrinous exudate and lesions of pleuropneumonia were identical with those studies in the field. Four goats were insufflated and inoculated subcutaneously in the neck with pleural exudate from that case. The dose given was 10 c. c. intranasally and 5 c. c. subcutaneously. Goat one developed a considerable oedema at the site of subcutaneous inoculation, and this abated within a week. Systemic reaction and fever were not noted until 25 days p. i. when early signs of pneumonia were seen, the temperature rising to 106°F. within a few hours and remaining high until death four days later. Pulmonary lesions identical with those occurring in the natural disease were seen on autopsy. Goat two developed a severe local oedema at the site of subcutaneous inoculation and within six days the neck and chest muscles were grossly infiltrated and swollen with exudate which collected in quantity along the lower borders of the lesion. During the next two weeks the lesion slowly retrogressed and appeared as a marble size induration 26 days p. i., finally disappearing 34 days p. i. The reaction was afebrile and marked by no systemic disturbance, but 68 days p. i. the animal was again visibly ill with high fever. Four days later, signs of pneumonia were seen, and the animal died after eight days' illness. The pulmonary lesions were again typical. Goat three reacted rapidly, inflammatory oedema commencing 24 hours p. i., and quickly involving the muscles of the neck, forelimb and chest. Fever commenced two days p. i. rising to 107°F. on the fourth day and dropping by lysis until the seventh day, when the condition of the animal necessitated destruction. No pneumonia was seen in this case at autopsy. Goat four did not react over a period of three months.

Five goats were subinoculated with local lesion exudate from goat three, immediately after destruction. Goats five and six were subcutaneously inoculated in the left thigh with 10 c. c. exudate, goat seven with 5 c. c. subcutaneously in the neck and goats eight and nine were intravenously inoculated with small fragments

of local lesion tissue, a wide bore cannula being used for this operation. Goat 5 showed oedema at the site of inoculation one day p. i., increasing daily until, at the tenth day when the animal was destroyed, the entire limb and hip were enormously swollen, and the animal dead lame. Fever commenced four days p. i., and the temperature was 105.6°F. for the next five days, dropping suddenly on the tenth day. No pulmonary lesions were seen on autopsy. Goat 6 reacted similarly, being destroyed at the tenth day, and no pulmonary lesions found. Goat 7 developed inflammatory oedema at the site of inoculation, but the lesion abated in extent by the twelfth day p. i. and steadily decreased thereafter until it was only visible as a beat size nodule at one month p. i. There were two sharp rises of temperature, one five days p. i., and the second at the seventeenth to nineteenth day p. i., but the condition of the animal was unaffected. No further reaction was observed over a period of two months. Goats 8 and 9 failed to react over period of ten weeks. Six more healthy goats were sub-inoculated with fresh local lesion exudate from goat 5. Goat 10, inoculated subcutaneously in the left thigh with 5 c.c. exudate, showed a sharp rise of temperature on the fourth day p. i. and fever up to 106.4°F. for the next three days and then returned to normal. Moderate oedema of the thigh muscles was seen seven days p. i. and this had disappeared ten days p. i. No further reaction was seen over the next two months. Goats 11 and 12 were insufflated with 5 c.c. exudate, using a de vilbis atomizer. In goat 12 a febrile reaction was seen thirteen to seventeen days p. i., the temperature swinging between 106°F.-107°F. Definite signs of pneumonia were seen twenty days p. i. and death occurred the following day, preceded by severe diarrhoea and a sudden drop in temperature. The pulmonary lesions were typical of the natural disease. Goat 11 did not react, and died of intercurrent disease ten days p. i. Goat 13 was insufflated and subcutaneously inoculated in the thigh with 5 c.c. exudate. The only reaction seen was a mild, transient oedema at the site of subcutaneous inoculation. Goats 14 and 15 were intravenously inoculated each with twelve oat-seed size fragments of the local lesion tissue of goat 5. Goat 14 did not react over a period of two months. Goat 15 also failed to react, and when destroyed one month later, the right lung showed two infarcts in the middle lobe. Goats 16 and 17 were each insufflated with 1-2 c.c. fresh pleural exudate from goat 11. The temperature of goat 16 commenced to rise one day p. i. attaining 106.4°F. four days p. i. On the sixth day p. i. a sudden drop in temperature coincided with diarrhoea, the animal dying the following day. Signs of pneumonia were seen on the third day and were advanced by the fifth day p. i. Typical lesions of pleuropneumonia were seen on autopsy. The history of goat 17 was similar but pulmonary lesions were not so advanced. Two c.c. pleural exudate (lymph virus) from goat 16 was insufflated in goats 18 and 19; in goat 18, fever commenced one day p. i. rising steadily to 106.5°F. four days p. i. and falling rapidly when diarrhoea set in twenty-four hours or so before death at the seventh to eighth days p. i. Pneumonia was definite at the fourth day and aggravated until death. Lesions of pleuropneumonia were characteristic and severe. In goat 19, the reaction was similar, this animal being destroyed nine days p. i. on a moribund condition. Lesions of pleuropneumonia were not so advanced as in the previous case, but were quite definite. Pleural exudate from goat 18 was insufflated in goats 20 and 21. It was evident at this stage in the transmission experiments that the

virus had become fixed in virulence for the type of goat used. Three goats then inoculated in the thigh and three subcutaneously in the neck with fresh lymph virus all reacted with fever and marked oedema at the site of inoculation, progressing to death, with typical lesions of pleuropneumonia. No variation in the virulence of the lymph virus, as tested by insufflation, was noted between local lesion exudate and pleural exudate. In all, forty-seven goats were insufflated. Forty-one goats reacted and died after a typical syndrome with specific lesions of pleuropneumonia at autopsy. Three showed a varying degree of reaction, but survived; two failed to react, and one died of intercurrent parasitic disease within a few days of inoculation. Thus, the percentage reaction was 93.6 with 87.4 per cent morbidity.

Inoculation of lymph virus subcutaneously in the ear tip of goats

Of 22 goats inoculated ear tip with a dose of virus varying from 0.1 to 1 c.c., none survived. The reaction in these goats was uniform. Severe oedema of the external ear, increasing daily, was seen twelve to twenty-four hours after inoculation, the temperature rising steeply to 106°F.-107°F. within the same interval, and remaining at this level during the eight to twelve days of illness. Pneumonia was usually evident at the fifth day, and death was ushered in by severe diarrhoea and a sudden fall in temperature to subnormal. In all cases, pulmonary lesions were most marked. A precisely similar result was obtained when a few drops of lymph virus were rubbed in on the lightly scarified skin of the ear tip of four healthy goats.

Transmission by blood

One healthy goat was inoculated subcutaneously in the neck with 2 c.c. blood from a routine virus producer goat at the height of reaction, and reacted fatally as if inoculated with fresh lymph virus.

Transmission by contact

Two healthy goats were placed in a shed approximately 10 ft. x 8 ft. x 12 ft. and, three days later, four healthy goats inoculated subcutaneously in the ear tip with 0.2 c.c. fresh lymph virus were introduced into the shed, allowing free contact between the animals. The inoculated goats succumbed within ten days. The first goat in contact was seen to be ill ninety days later and developed symptoms of the disease which terminated fatally in ten days. The pulmonary lesions were mild but definite in this case and the causal organism was isolated from the lesions. The history of the second animal was similar, signs of illness being seen 94 days p.i. Pneumonia with high fever was evident six days later and signs of recovery at the twelfth day of illness when, with the onset of diarrhoea the temperature fell steeply but remained normal for the next seven days. The goat was destroyed in this phase, and showed early lesions of pleuropneumonia, the organism being isolated from the lesions.

In a repeat test, a healthy goat was insufflated with lymph virus and tied in a shed alongside a healthy goat. The inoculated goat reacted typically and died of pleuropneumonia 12 days p.i. Fever was noted in the test goat on the twelfth day, rising to 106.5°F. on the sixteenth day and swinging between 105°F.-106.5°F. during the thirteen days illness. Pneumonia was evident on the sixth day of illness,

Advanced lesions of pleuropneumonia were seen on autopsy. In a further test, a healthy goat was insufflated with lymph virus and tied in the middle of large, airy shed approximately 40 ft. \times 20 ft. \times 20 ft. Seven days later, two healthy goats were tied in the shed, one 20 and the other 10 feet away from the inoculated goat, separate feeding and watering being provided for each animal. The inoculated goat died of pleuropneumonia in twelve days. The first contact goat showed a steady rise in temperature up to 106.5°F. from the fifth to eighth day of observation, pneumonia on the ninth and death on the eleventh day. Lesions of pleuropneumonia were seen on autopsy. The history of the second goat was similar, death also occurring on the eleventh day. Infection lingers in sheds for at least one month (longer period not tested) after the last fatal case. On two occasions healthy goats were placed in such sheds pending experiments, and the disease swept through both batches. It became vitally necessary, therefore, to construct sheds which could be adequately disinfected between experiments.

Reaction of the virus in sheep

Seven sheep were inoculated subcutaneously in the neck each with 5 c.c. fresh lymph virus. Three sheep did not react. Three developed a marked thermal and local reaction similar to that seen in goats inoculated in the same manner; death occurred four, five and six days p. i. The lungs and internal organs were acutely congested and haemorrhagic gastro-enteritis was present. No local reaction was seen in the fourth sheep, but the temperature rose to 106°F. four days and remained high until death twenty-one days p. i. This animal went down steadily in condition without clinical signs of illness. Both lungs were normal on autopsy. The pericardial sac and the mesentery were distended with a yellowish, fibrinous exudate. A healthy goat inoculated subcutaneously in the ear tip with a small dose of this material reacted as vigorously as if inoculated with lymph virus. Finally five sheep were insufflated with fresh lymph virus. Three did not react. The fourth sheep showed a transient thermal reaction. The reaction in the remaining sheep was, however, severe and death occurred on the sixteenth day, pneumonia being evident on the twelfth. Lesions of acute pleuropneumonia with exudative peritonitis and haemorrhagic gastro-enteritis were seen on autopsy.

Reaction of the virus in cattle

Two hill bulls were subcutaneously inoculated at the point of the shoulder with 10 c.c. fresh lymph virus and insufflated at the same time with 5 c.c. of this material; neither reacted over a period of one month. A third hill bull and a buffalo calf were subcutaneously inoculated with 5 c.c. saline pneumonic lung mash. Some inflammation was seen at the site of inoculation in both animals and this was ascribed to secondary infection, as active suppuration supervened.

Reaction of the virus in laboratory animals

Three rabbits were subcutaneously inoculated at the side of the neck with 1 c.c. fresh lymph virus; no reaction ensued over three weeks observations of three guinea-pigs subcutaneously inoculated with 0.5 c.c. of the same virus one developed fever on the eighth day p. i. lasting for a week, thereafter returning to normal.

Nature of the virus

(a) *Tenacity*.—Lymph virus (pleural exudate and saline lung mash) from routine virus producers was stored in sealed ampoules at 5°C. and tested for virulence in healthy goats at varying intervals of storage. Four goats insufflated with four days stored virus reacted typically and died with specific lesions of pleuropneumonia on autopsy, the virus being recovered from the lesions. The same results were obtained in three goats insufflated with seven days stored virus, two goats inoculated subcutaneously in the ear tip with 0.25 c.c. 15 days stored virus, and in two goats similarly inoculated with one month stored virus. Six goats were subcutaneously inoculated in the neck each with 3 c.c. three months stored virus. Swelling at the site of inoculation was absent in all six animals but four developed considerable inflammation of the prescapular gland on the side of inoculation. A standard thermal reaction was evoked in five goats and pneumonia was seen seven to ten days p.i. with death at nineteen, sixteen, eleven, sixteen and seventeen days respectively p.i. Lesions of pleuropneumonia were encountered in these five goats, the virus being recovered. The remaining goat showed a mild rise of temperature on the sixth to seventh day p.i. and no further reaction over a period of thirty-six days. Five goats were each inoculated subcutaneously with 2 c.c. virus stored for three and a half months. There was a slight, transient swelling at the site of inoculation in all five goats. One animal developed fever seven days p.i. and this persisted until death fourteen days p.i. pneumonia was seen nine days p.i. and typical lesions of pleuropneumonia were seen on autopsy.

(b) *Filtrability*.—Five c.c. fresh lymph (pleural exudate) was diluted $\frac{1}{2}$ with peptone broth, buffered with M/15 phosphate, and passed through a Seitz F. K. disc at moderate positive pressure. Fresh lymph virus has a constant pH 7.4 and no adjustment before buffering is really necessary. Three healthy goats were insufflated each with 5 c.c. filtrate. All three goats reacted as if given pure lymph virus, and characteristic lesions of pleuropneumonia were seen in each animal on autopsy. As would be seen later, the casual organism of the disease was originally isolated in purity from such Seitz filtrates, thus giving an adequate control to the filter discs used. The same result was obtained in five goats subcutaneously in inoculated in the neck with lymph virus, diluted $\frac{1}{2}$ peptone broth, buffered and passed through Berkefeld V candle at a negative pressure of 15 mm. mercury. Graded filters were not available to test the limits of filtrability.

Other strains of virus

In addition to the strain of virus isolated from an outbreak of contagious pleuropneumonia of goats in Bombay Province, a strain was isolated from an extensive outbreak of the disease in the army depôt at Benares (U. P.) and another strain from the disease in goats in the North West Frontier Province. Both these strains behaved in precisely the same way as the original Bombay strain when insufflated or subcutaneously inoculated in healthy goats at Izatnagar. As will be seen later batches of goats vaccinated at Benares and in the North West Frontier Province with the Bombay strain virus were strongly protected against the local Benares and North West Frontier Province strains. Further, a sample of pleural exudate was received through the Veterinary Investigation Officer,

Assam, from an outbreak of pleuropneumonia of goats in that province. Two healthy goats each received 10 c.c. of this material subcutaneously in the neck and a typical local and thermal reaction ensued in both animals, which recovered. These animals proved immune to test two months later with the Bombay strain of virus, the two controls to the test reacting fatally. The Veterinary Investigation Officer, Assam, was instructed to inoculate healthy goats subcutaneously with fresh pleural exudate from typical field cases and obtained results parallel to those seen in goats inoculated at Izatnagar with the Bombay, Benares and the North West Frontier Province strains. No results were obtained in several attempts to isolate the Madras strain of virus in goats at Izatnagar with materials sent by the Veterinary Investigation Officer, Madras. The indication was that the material (pleural exudate) was inert after five days in transit, as the original sample was fully virulent when tested in goats at Madras. The Veterinary Investigation Officers, Sind and the Central Provinces, also reported outbreaks of pleuropneumonia in goats and were, on instruction, able to reproduce characteristic thermal and local reactions in healthy goats inoculated subcutaneously in the neck with fresh pleural exudate from typical field cases, and the specific pathological features of the local and pulmonary lesions were identified at Izatnagar. Moreover, the Veterinary Investigation Officer, Sind, succeeded in the serial transmission in goats of the virus isolated. Attempts at the isolation of the Central Provinces and Sind strains of the virus failed at Izatnagar, the viruses obviously having perished in transit. Investigation into an outbreak of pleuropneumonia of goats in a large breeding farm at Etah (U. P.) yielded data of considerable interest. Between 2 April and 31 May, 1943, 121 out of a total of 190 goats on the farm had contacted the disease; of this number, 75 died, seven were destroyed in extremis, 22 recovered, whilst 17 were ill and segregated. Preliminary investigation by the Veterinary Investigation Officer, United Provinces, indicated a strain of *Pasteurella* as the aetiological factor. We failed to isolate a *Pasteurella* strain in 11 cases bacteriologically examined, but an organism provisionally classified as an *actinobacillus* was isolated from the penumonic lung of seven of these cases; this organism growing readily on agar slant and in ordinary broth. Cultures evoked a virulent septicaemia when intravenously inoculated in goats and rabbits, but subcutaneous inoculation of such animals was without effect. Also, goats liberally insufflated with freshly isolated broth culture failed to react. The disease could be readily transmitted to healthy goats at Izatnagar by insufflation and subcutaneous inoculation with pleural exudate of material obtained on autopsy from the eleven cases referred to, and in such experimental animals there was no further evidence of the organism isolated from the original field cases. The virus so established from the field cases was serially passaged by insufflation in healthy goats at Izatnagar, and the resultant syndrome and lesions were as seen in goats used in the passage of the Bombay strain of virus.

EXPERIMENTAL DISEASE-PATHOLOGY

The following lesions were seen in a series of 15 cases of the experimental disease induced by insufflation with the Bombay strain of lymph virus.

Goat 1.—Died 27 days p.i., period of illness five days. Pneumonia right and left lungs; diaphragmatic lobes consolidated and adherent to chest wall

and diaphragm, with a few small patches of well-organised pleurisy. Right middle lobe, infraacts of various size and some interstitial oedema with scanty, clear fibrinous exudate in the chest cavity.

Goat 2.—Died 20 days p.i., ill 14 days. Apical and diaphragmatic lobes of both lungs acute primary congestion, no pleurisy or exudate in chest cavity. Pericarditis, endocardium petechiated.

Goat 3.—Died 19 days p.i., ill 6 days. Apical and middle lobes right lung consolidated and covered with a thick layer of coagulated fibrin and loosely adherent to chest wall; marked interstitial oedema and 'marbling'. On the left side, apical lobe consolidated and 'marbled' and diaphragmatic lobe acute primary congestion. About 200 c.c. exudate in chest cavity. No pericarditis, but definite myocarditis.

Goat 4.—Died 11 days p.i., ill 4 days. Acute primary congestion diaphragmatic lobe, slight congestion and interstitial oedema middle lobe left side; about 40 c.c. exudate in chest cavity. Pericarditis with effusion similar to that in chest cavity.

Goat 5.—Died 76 days p.i., ill 9 days. Apical and middle lobes right side consolidated. Haemorrhagic infraacts of varying size in diaphragmatic lobe same side and in all lobes of left side; about 5 c.c. exudate in chest cavity. Pericarditis.

Goat 6.—Died 21 days p.i., ill 8 days. Entire left lung with the exception of posterior third diaphragmatic lobe consolidated; gross generalized pleurisy, the lungs being firmly attached to the chest wall and heart sac; interstitial oedema well marked. Posterior half of diaphragmatic lobe, right side, consolidated and 'marbled', but no pleurisy. Haemorrhagic infraacts in both lungs. No exudate in chest cavity.

Goat 7.—Died eight days p.i., ill three days. Tip of apical lobe right side and left middle lobe acute primary congestion and interstitial oedema. No pleurisy or exudate in chest cavity.

Goat 8.—Died seven days p.i., ill four days. Apical lobe and anterior part middle lobe right side consolidated, 'marbled' and coated with a thick layer of coagulated fibrinous exudate; costal pleura both sides extensively petechiated. No exudate in chest cavity. Pericarditis.

Goat 9.—Died eight days p.i., ill four days. Apical lobe and anterior half middle lobe right side consolidated and covered with coagulated fibrinous exudate. Lesions similar left side but no pleurisy. 'Marbling' distinct in both lungs and haemorrhagic infraacts in apical lobes both sides; about 500 c.c. exudate in chest cavity.

Goat 10.—Died 14 days p.i., ill 8 days. Lesions confined to right side, apical and anterior border middle lobes consolidated, and affected parts covered with thick layer coagulated fibrinous exudate. No exudate in chest cavity.

Goat 11.—Died eleven days p.i., ill seven days. Apical lobe and anterior part middle lobe right side consolidated and 'marbled' with overlying pleurisy and adhesions to the chest wall haemorrhagic infarcts apical lobe; about 3 c.c. exudate in chest cavity. Pericarditis.

Goat 12.—Died eighteen days p.i., ill six days. Apical lobe and anterior border middle lobe right side consolidated; several haemorrhagic infarcts in diaphragmatic lobe; no pleurisy. Left lung unaffected; about 40 c.c. exudate in chest cavity. Pericarditis.

Goat 13.—Died ten days p.i., ill six days. Right side, apical lobe consolidated and acute primary congestion middle and diaphragmatic lobes with several haemorrhagic infarcts. Entire left lung acute primary congestion with infarcts. No pleurisy. Chest cavity contained about 5 c.c. exudate.

Goat 14.—Died ten days p.i., ill six days. Apical and middle lobes right side consolidated and 'marbled' haemorrhagic infarcts in diaphragmatic lobe; no pleurisy. Left apical and middle lobes acute primary congestion with patch pleurisy and early adhesions easily broken down; about 100 c.c. exudate in chest cavity. Pericarditis.

Goat 15.—Destroyed nine days p.i., ill six days. Apical lobe right side consolidated and haemorrhagic infarcts diaphragmatic lobe. Pleurisy with adhesions confined to apical lobe. Lesions in left lung similar. Scanty exudate in chest cavity. Pericarditis.

Summing up, bilateral pneumonia was seen in nine out of fifteen cases, with no predilection for any particular lobes. If anything the anterior lobe was most frequently affected (13 cases), more usually in conjunction with the middle lobe and less frequently the diaphragmatic. 'Marbling' of the lungs was seen in six cases and interstitial oedema, the precursor of this lesion, in three cases. The clear, amber-coloured rapidly clotting exudate seen in the chest cavity of field cases was seen in ten cases of the series, but in only two cases was it particularly noticeable; in five of these cases the amount present was less than 5 c.c. Pleurisy was seen in eight cases, and in six of these the lesion extended over the affected portions of the lungs and was not generalized on the side of the chest affected; in two cases, pleurisy was patchy. Early organization with adhesions was the rule. In six out of seven cases where pneumonia was bilateral, pleurisy was unilateral. Thrombosis of branches of the pulmonary artery was a common feature, and haemorrhagic infarction was seen in eight cases; in six of these, they occurred in lobes of the lung unaffected with pneumonic change. Pericarditis with effusion, the exudate being similar to that encountered in the chest cavity, was seen in eight cases and was an obvious extension of pleurisy; in only one case was the heart muscle affected. Cut slices of the pneumonic lung showed little, if any, variegation of the lobules as seen in contagious bovine pleuropneumonia. Oedema of the bronchomediastinal glands and the mediastinum was a constant feature. The bronchioles contained a frothy exudate, but the bronchi and trachea, pharynx and larynx were unaffected. Nasal discharge and rhinitis was a constant lesion.

The lesions in goats inoculated with lymph virus subcutaneously in the tip of the ear differ little from those described in the foregoing series. The disease so

induced is often more rapid in its course, and somewhat septicaemic in character. In a few cases, pneumonia may be absent on autopsy and the lesions more suggestive of a pure septicaemia. That the virus can invade the blood stream has already been shown, but no attempt has been made to demonstrate it in the parenchymatous tissues other than the lung; it is possible that the tissues harbour the virus by way of a septicaemic generalisation. Over 400 goats were used in the experimental work on contagious pleuropneumonia of the goat, and this number was necessitated by the frequent casualties due to intercurrent disease through which set experiments were nullified and had to be repeated, often more than once. The most formidable cause of loss was parasitic disease. On no occasion was parasitic invasion of the lung seen, but parasitosis of the gastro-intestinal was uniform and grievous in its incidence. All the known parasites were present in varying concentrations—usually high—in individual goats; the assessment of body weights of these animals gave a shrewd estimate of the general malnutrition caused by parasitic infestation. These animals had a spurious vitality and balance of health readily upset by adverse weather, or, worse still, by being subjected to experimental inoculations which had an immediately devitalising influence, latent parasitic disease thus gaining the upper hand. In all, approximately 38 per cent of goats used succumbed to parasitic disease. 'Pimply gut' was the most serious of these conditions, and, in a considerable proportion of cases, evidence was obtained to show that a certain high incidence of purulent peritonitis was related to this infestation acting in conjunction with *C. pyogenes*.

Anatomy and histopathology

The lesion in the lungs is a pneumonitis, or, more specifically stated, a primary pulmonary lymphangitis. The term trabecular lymphangitis or perilymphangitis would be more expressive of the genesis of the lesion, and the term 'septal' pneumonia strictly correct according to modern classification of the pneumonias. These septal pneumonias, either purulent, fibrinous or productive, are not uncommon in various animal species in which the septal tissue of the lungs is particularly well developed, with an abundant free communication with bronchi and pleura. They originate in peribronchial and pleural tissue and extend along the vascular and lymphatic channels of the septa.

Whilst this is the fundamental nature and origin of the specific pneumonic lesions in contagious pleuropneumonia of the goat, the pneumonic lobes show the ordinary features of hepatization, the type of pneumonia being mixed croupous-catarrhal, originating apparently from a bronchiolitis. Necrosis of lobules has not been seen. The septal veins are intensely engorged, and stasis of the venous system is indicated by the presence of blood pigment within the veins. The infarcts in the lung follow the classic pattern, i.e., an area in which the veins are stuffed with blood whilst the surrounding tissue shows an 'apoplectic' haemorrhage. Swelling and proliferation of the endothelium with some hyperplasia of the wall is seen frequently in branches of the pulmonary artery with thrombosis of a few of the smaller terminal branches. It is well established, however, that thrombosis of branches of the pulmonary artery is not in itself a sufficient cause for the development of infarcts and that a deranged and sluggish circulation is the essential

contributory factor. This syndrome is well seen in contagious pleuropneumonia of the goat. The wedge shaped infarcts are plum coloured, and only moderately raised above the pleural surface; they are easily differentiated from a bronchopneumonic focus by the fact that the periphery of the lesion is not surrounded by zones of atelectasis and emphysema, and further that the cut surface is smooth and not granular and bleeds somewhat freely when the infarct is quite recent. The perilobular, perivascular and peribronchial tissue is distended with exudate containing numerous histiocytes, lymphocytes and polymorphs. Histiocytes derived from the perivascular and peribronchial stroma infiltrate along the lines of the perilobular stroma and aggregations of these cells are seen at interlobular angles. The potential septal spaces are increasingly infiltrated until the wedges of new cellular tissue encroach upon groups of alveoli already pneumonic. So great is the pressure, combined possibly with some amoeboid power of these cells, that they migrate into the air spaces apparently through the septal lacunae (pores of Kohn). This process is usually focal in distribution and several pieces of the affected lung may have to be examined to gain a clear picture of the histological change involved. Breaking down of septal barriers and replacement of alveolar tissue with masses of histiocytes may advance to the stage of obliteration of quite large area of tissue. In some Madras specimens, tumour like masses of cellular tissue were seen in which all outline of the air spaces were lost; the bronchioles, resisting encroachment to the last, appeared as compressed slits lined by cuboidal epithelium.

It has been noted that myositis is a cardinal lesion surrounding the area of inflammatory oedema induced by the subcutaneous or intramuscular inoculation of lymph virus in healthy goats. This lesion of the muscle is strictly comparable developmentally and pathologically, with that evoked in susceptible cattle inoculated subcutaneously in the neck with a few drops of lymph virus (pleural exudate) from a case of contagious bovine pleuropneumonia. The connective tissue of the muscle is greatly infiltrated with a straw coloured, rapidly clotting, fibrinous exudate which freely drains from the muscle tissue on incision. In lesions of some standing, the exudate undergoes organization and the cut surface of the muscle is distinctly 'marbled' and the muscle bundles are widely separated by swathes of granulomatous tissue. Acute inflammation of the muscle bundles is succeeded by necrosis. Thrombosis of arterial branches is a specific feature of the lesion which is one of considerable diagnostic importance.

Isolation of the causal organism

It was fairly evident from a study of the pathological lesions, particularly of the local lesion induced by the subcutaneous inoculation of lymph virus in healthy goats, that an organism of the pleuropneumonia group was associated with the syndrome of pneumonia in the goat, if not aetiologically responsible. The results of filtration experiments, in which the infective agent in lymph virus was shown to pass Seitz E. K. and Berkefeld V filters, naturally led to the search for a filtrable organism. Isolation was attempted in Bennett's broth with 10 per cent added goat serum. The first attempts, sowing tubes of this medium with a few drops of Berkefeld V filtrate of local lesion or pleural exudate, gave indefinite results, but, later, as the virulence of the lymph virus enhanced and became fixed, it proved

simple and certain to obtain primary cultures with such filtrates. However, the organism could not be isolated from samples of virus stored for three days or more at 5°C. even if such material gave positive results when fresh. The indication was that the organism was filtrable only at certain stages of its development. The organism was first isolated from Seitz filtrates of blood from a goat insufflated with lymph virus and also from pericardial effusion of a second goat inoculated in the same manner. The organism could, however, be isolated more simply and directly from fresh lymph virus by a method originally described by Ebert and Peretz [1929] and quoted by Curasson [1936]. A thin sterile strip of filter paper is lightly dipped in lymph virus so that only a mere fraction is absorbed by the paper. Segments of the paper are cut, with sterile scissors, from and above the level at which moistening is apparent, and these are sown in the special medium used for the isolation of the pleuropneumonia organism. This method owes its success, it is claimed, to the more rapid absorption of the pleuropneumonia organism when present along with adventitious organisms, the latter absorbing more slowly. Whether this be the correct explanation, the method is of use in the absence of filter candles and is one that could be employed in the field.

The morphological and cultural characters of the organism of goat pleuropneumonia are similar to those of the organism causing contagious bovine pleuropneumonia. Whereas the bovine organism grows best in Bennett's broth with 10 per cent added horse serum, Bennett's broth with 10 per cent goat serum proves most satisfactory in the isolation and maintenance of cultures of the organism of pleuropneumonia of the goat. Bennett's broth with 10 per cent added bovine serum was much less satisfactory, and the same medium with 10 per cent horse serum was definitely inhibitory. The organism of goat pleuropneumonia possesses considerable tenacity in culture. Six tubes of Bennett's broth with 10 per cent added goat serum were sown with a fourth generation culture of the goat pleuropneumonia organism and incubated at 37.5°C. for three days when growth was well established. The plugs were then sealed with molten wax and the tubes stored at +5°C. Subcultures from these tubes were made at ten to fourteen days intervals in fresh tubes of Bennett's broth with 10 per cent added goat serum. The cultures of the goat pleuropneumonia organisms stored at +5°C. were viable at 275 days but not at 285 days. The classification of the goat pleuropneumonia organism as *Bordetella peripneumoniae capri*, as tentatively proposed by Longley [1940] to describe forms of an apparent pleuropneumonia organism seen by him on 'dark ground' examination of pleural exudate from cases the disease in Madras, seems therefore appropriate. The proof that the organism that we have isolated is the causal agent of contagious pleuropneumonia of the goat in India is that virulent and recently isolated cultures whether insufflated or subcutaneously inoculated consistently reproduced the disease in healthy goats and that the organism can uniformly be reisolated from the lesions produced.

Immunity

Whether a natural attack of the disease protects against a further attack, or whether recovery from the disease is absolute is not known; the latter is highly improbable in view of the nature of the lesions. Workers in Africa have stated that

recurrence of the disease may occur after apparent recovery, but nothing is known of this aspect of the disease in India. Our experiences in field investigation have shown that the level of susceptibility of goat populations to the experimental disease varies; thus, the 'takes' in goats at Izatnagar subcutaneously inoculated in the ear tip with virulent lymph 'virus' (Bombay strain) was 100 per cent whereas, accordingly to Longley [1940], goats in Madras Province do not react to this inoculation with the Madras strain of virus. We found that goats in the North-West Frontier Province held an intermediate position in order of susceptibility. Thus, in the original isolation of the North-West Frontier Province strain of virus in Peshawar goats, 13 goats were used. Of these, four inoculated subcutaneously in the ear tip with 0.25 c.c. fresh 'virus' from a local case of the disease destroyed in extremis failed to react; only three of six goats insufflated with the same 'virus' reacted with a typical syndrome and lesions; three remaining goats were inoculated intramuscularly in the thigh and of these only one reacted and succumbed to pleuropneumonia. When the North West Frontier Province strain of 'virus' was serially passed in goats at Izatnagar, it behaved precisely as the original Bombay strain. Next was studied the possibility of immunity in Izatnagar goats which had reacted to inoculation with the Bombay strain virus and survived. The results obtained in the immunity tests of these animals were necessarily equivocal on account of potential variations in susceptibility, yet indicative of the general trend. The question of the susceptibility ratio in goat populations is obviously of great importance in the general bearings of immunity and particularly in the application of methods of conferring immunity. It is equally obvious that a method of proved value in one province may not be applicable in another province. It might be argued that the method with the highest level of safety compatible with an effective immunity is the requisite criterion and one that could be universally applied. However, we would prefer to advocate the use of virulent 'virus' vaccines just within the limit of safety; this is based on our experience of immunity in the analogous disease in cattle.

The indication of immunity in goats after recovery from a severe reaction evoked by the Bombay strain 'virus' given parenterally is seen in the following cases. Goat 1 given 2 c.c. fresh lymph 'virus' subcutaneously in the neck reacted severely, eventually recovering only to die when tested for immunity by insufflation with homologous strain virulent lymph 'virus' 61 days after the original inoculation. Typical lesions of pleuropneumonia were seen in this goat and in the two controls used; the degree of immunity was nil, the animal under test reacting as severely as the controls. Three goats which reacted to and survived inoculation of virulent Bombay strain 'virus' in the thigh muscle were tested for immunity in the same way; two goats were tested 52 days and the third 42 days after the original inoculation; one of the pair and two controls died of typical pleuropneumonia after a severe reaction and two were solidly immune, with complete absence of reaction to the test dose. Two survivors of the series of insufflated 'virus' producers which reacted severely to the original inoculation survived the same immunity test 18 and 28 days later, both controls dying of the typical disease. There is, therefore, some evidence that a measure of immunity is gained by an animal which survives the experimentally produced disease. The immunity is, however, equivocal. Although no reaction to the test dose was seen in

the goats that proved immune to the test, four of six goats, the reaction in the remaining two was so severe as to indicate no immunity whatsoever. There was an indication, from the original reaction to the virus, that all six goats had a fairly uniform level of susceptibility and one would, therefore, have expected uniform results in the immunity test of these six animals. The anomalous results of the test cannot be explained but a similar phenomenon has been encountered in our studies on immunity in the analogous disease of cattle.

Longley [1940] showed that goats in Madras Province acquired a substantial immunity when given 5 c.c. virulent Madras strain 'virus' subcutaneously in the ear tip; the morbidity rate in goats so vaccinated was 5.4 per cent compared with 75 per cent in unvaccinated controls. We have shown that this method of inoculation, using much smaller doses of virulent Bombay strain virus, is attended with 100 per cent mortality in goats at Izatnagar. Thus, this method of vaccination is not one that could be recommended for general use in the field. Its employments would, apparently, depend on the susceptibility level of the goat populations to be protected; and useful as it might be in certain areas, it is essential to have a more attenuated vaccine for the protection of more susceptible goat populations. Formalinized vaccine, in which the virus is inert, has been recommended by Longley [1940]. Eighty-three per cent of goats vaccinated with formalinized vaccine in Madras were immune when tested by insufflation with fully virulent 'virus' 14 days after vaccination. These results have not been confirmed in goats at Izatnagar. Thus, 12 goats were inoculated subcutaneously in the neck each with 4 c.c. formalinized vaccine prepared from the Bombay strain 'virus' according to Longley's formula. Six of these goats died of intercurrent disease, chiefly purulent peritonitis, leaving six goats available for test by insufflation 14 days after vaccination. All six reacted fatally and as vigorously as the control, thus indicating a complete absence of immunity. The test was repeated in eight goats, each given 5 c.c. vaccine. The immunity test dose was 0.25 c.c. 15th generation culture given subcutaneously in the neck 34 days after vaccination. The control gave an average reaction, but six of the eight goats tested reacted very severely, and the lesions were of the septicaemia type. The indication was that hypersensitiveness and not immunity followed vaccination. The two remaining goats reacted moderately and survived.

In contagious bovine pleuropneumonia immunity is best established with attenuated culture vaccines, and the next obvious step was to determine whether this method could be applied in the control of the analogous disease. The Bombay strain of the organism was subcultured at seven to ten day intervals in Bennett's broth containing 10 per cent goat serum and the possible attenuation of later generation cultures tested in susceptible goats by the ear tip route. Four goats each inoculated with 0.5 c.c. 18th generation culture reacted severely with inflammatory oedema at the site of inoculation and a typical febrile reaction; three died of pleuropneumonia and one survived. Five goats tested in the same way with 33rd generation culture reacted less severely with oedema and fever, and although all died within a week, none developed pleuropneumonia. An appreciable attenuation in virulence was discerned in cultures at the 43rd generation. Five goats were

inoculated each with 1 c.c. of this culture subcutaneously in the ear tip, the inoculation being reactionless in all five goats. Goat 1 died 33 days p.i., following an abortion. Goat 2 died of parasitic gastro-enteritis 10 days p.i. Goat 3 was tested for immunity 42 days p.i., the test dose being 1 c.c. virulent lymph 'virus' subcutaneously in the neck; a very slight local and thermal reaction ensued and abated rapidly, two controls to the test reacting severely and fatally. A mild febrile reaction was seen five to nine days p.i., in goat 4, and the temperature rose again 16 days p.i. and fluctuated between 105-106 °F. until signs of pneumonia were evident forty-eight days p.i. No reaction to the test dose was seen in goat 5 which succumbed to parasitic gastritis 24 days p.i. Five more goats were given 1 c.c. 43rd generation culture subcutaneously in the tail tip. Goat 1 showed no reaction and was tested for immunity 51 days p.i. with 1 c.c. lymph virus subcutaneously in the neck; a mild and transient local reaction was seen, but no thermal reaction. This animal eventually died of parasitic disease 65 days p.i., and the lungs showed no lesions. Goats 2, 3 and 4 did not react to the inoculation and died of parasitic disease 15, 25 and 13 days p.i., respectively. Goat 5 showed a sharp rise of temperature up to 106°F. from the 23 to 26 days p.i., and was thereafter normal until tested for immunity thirty-nine days p.i.; beyond a slight transient swelling at the site of inoculation, there was no reaction to the test dose. The two controls used in the immunity test died of pleuropneumonia after a severe reaction. The indications from the tests were that the 43rd generation culture was attenuated in virulence, and caused no reaction, either local or systemic, when inoculated subcutaneously in the ear tip or tail tip of susceptible goats. Unfortunately, three of five goats of the ear tip series died of intercurrent parasitic disease, no lesions being seen in the lungs of these animals, and one died of pleuropneumonia, in spite of the fact that there was no outward reaction to the virus. The remaining goat proved solidly immune on test, surviving for several months until it was discontinued from experiment. In the second series inoculated tail tip the results were again marred by the deaths of three goats from parasitic disease, but the remaining two goats were solidly immune. There was, therefore, some indication that the 43rd generation culture was of some immunizing value, but the fact that one of ten goats vaccinated ultimately died of pleuropneumonia following vaccination points to a certain danger in the use of the culture, although, in our experience, one must always anticipate the hypersusceptible goat and assess results accordingly.

Fifteen healthy goats were then tested with 48th generation culture, 0.5 c.c. given subcutaneously in the ear tip; earlier work showed this generation of culture to be uniformly lethal when given subcutaneously at the side of the neck in susceptible goats. In only one of the vaccinated goats was any reaction seen and this was transient and mild. Four goats of the series were tested for immunity 16 days later with 0.5 c.c. virulent lymph subcutaneously at the side of the neck. Three reacted as if inoculated with fresh virus and died thirteen, six and six days p.i., and the reaction in the fourth goat was relatively mild with rapid recovery. This animal died 36 days p.i., from parasitic disease; no lesions were found in the lungs of this case which was, therefore, considered to have a moderate measure of immunity. Four goats of the series were tested in the same way, 28 days p.i., with 0.25 c.c. virulent lymph. One goat showed a slight local reaction to

the test dose and reaction was absent in the second goat, and both were discontinued three months p.i. The first goat was considered still mildly susceptible to the 'virus', and not altogether immune, and the second goat strongly immune. Two remaining goats and two controls died of pleuropneumonia 10 and 16 days p.i., after a severe reaction, and showed no immunity whatsoever. The last seven goats of the series were tested by insufflation with virulent lymph 31 days p.i. Five of these goats did not react to the test dose; three were discontinued as solidly immune three months p.i.; and two died of parasitic disease 19 and 15 days p.i. The remaining two goats with two controls reacted with typical pleuropneumonia. There was thus some indication that a degree of immunity is conferred on susceptible goats vaccinated with an attenuated culture which proves safe in the majority of goats vaccinated. But the degree of immunity is not comparable with that conferred in equally susceptible cattle vaccinated in an extremity (tail tip) with an attenuated culture of the bovine pleuropneumonia organism which proves lethal when given subcutaneously, at the same dose, in the neck.

Later generations of culture were then tested. Three healthy goats were each subcutaneously inoculated in the ear tip with 0.5 c.c. 71st generation culture and no reaction ensued; the immunity test was 0.5 c.c. 8th generation cultures subcutaneously in the neck 25 days p.i. One goat showed a mild local and thermal reaction and recovered uneventfully; the other two goats did not react and all three were discontinued 55 days p.i., as solidly immune; the control reacted fatally with lesions of pleuropneumonia. Seven goats were vaccinated in the same manner each with 74th generation culture and tested for immunity 20 days later each with 0.25 c.c. 8th generation culture given subcutaneously in the neck. None of these goats reacted and were eventually discontinued as solidly immune; the control reacted severely and died of pleuropneumonia. Finally, ten goats were vaccinated subcutaneously in the ear tip with 0.5 c.c. 80th generation culture. A rather severe local and febrile reaction was seen in two goats, one of which died of typical pleuropneumonia 36 days p.i. A mild local and febrile reaction, rapidly abating, was seen in two more goats, but one died of *Corynebacterial* pleurisy 14 days p.i. and it could not be decided whether this septic infection was primary or superimposed on a *Borrelomyces* infection. Two more goats showed a mild and transient local reaction. Thus, the reaction in the series inoculated with 80th generation culture was somewhat critical in comparison with the series inoculated with the 74th generation culture. A possible explanation is that the quality of goats during the later stages of the work was extremely poor, due to prevailing scarcity of these animals, and the inherent debility of the goats used was noted, throughout experiments, to be a factor predisposing to untoward or unpredictable reactions. The eight survivors of the series vaccinated with 80th generation culture were tested for immunity with 0.3 c.c. 15th generation culture given subcutaneously in the neck 37 days after vaccination, along with one control. Seven goats were wholly immune to the test and these were discontinued 34 days later; the eighth goat did not react to the test dose but died 21 days later of extensive parasitic debility and bilateral apical pneumonia considered terminal and not specific in type. The control reacted and died of pleuropneumonia six days p.i.

Four survivors to the immunity test of the series vaccinated ear tip with 1.0 c.c. 71st generation culture were retested for immunity, six months after the first

immunity test, with 0.5 c.c. 7th generation culture given subcutaneously in the neck; one of the animals under test showed a slight thermal reaction of five days duration and all were discontinued as solidly immune 18 days after the test dose, the control dying of pleuropneumonia. Similarly, four survivors of the immunity test of the series vaccinated ear tip with 0.5 c.c. 48th generation culture were retested for immunity. Three of these animals and one control were insufflated with virulent lymph 'virus' three months and eight days after the first test; one showed a mild thermal reaction lasting for 10 days, the control reacting fatally. These animals were again tested for immunity six months and five days after the preceding test, the test dose being 0.25 c.c. 13th generation culture subcutaneously in the neck. One goat showed a mild local and thermal reaction and a second goat a severe local and mild thermal reaction lasting for six days, and all three were discontinued two months later; the control died of pleuropneumonia. The fourth goat was retested for immunity three months and eleven days after the first test with 0.25 c.c. 8th generation culture subcutaneously in the neck. There was no reaction and the control reacted fatally. This animal was again tested with 0.25 c.c. 13th generation culture, given as before, seven months after the preceding test and showed a decided local and thermal reaction of six days duration and was discontinued one month later as immune; the control died of pleuropneumonia.

The 45th generation culture was put to a field test in the North West Frontier Province. Fifty-seven goats were vaccinated ear tip with 0.5-1.0 c.c. culture and placed in an open pen. None reacted to the vaccination. At the time of selection of these goats, several were weeded out as obvious cases of pleuropneumonia, and two of the animals selected as healthy and fit for test died within the next few days, showing that they were in the incubative stage at the time of vaccination. During the ensuing two weeks, five more goats succumbed to pleuropneumonia and, thereafter, there were no more deaths due to this cause. The vaccine seemed effective in cutting short further losses. Since it is well established in the North West Frontier Province that once the disease makes its appearance in a herd, it progresses steadily until the greater part of the herd is wiped out (90 to 100 per cent mortality).

Unfortunately, during the six weeks that the vaccinated goats were under observation, 30 died of extraneous disease, chiefly parasitic. This left only 20 goats available for immunity test which was conducted by placing a batch of infected goats along with the vaccinated goats within the pen. The infected goats died within a few days and none of the vaccinated goats showed any sign of having contracted the infection after a month's further observation. Thus, it would appear from this test that (a) vaccination was instrumental in limiting the spread of the disease in an already infected herd and (b) the standard of immunity conferred by the vaccine was approximately 90 per cent. The possibilities of vaccination in the field were examined at an army goat depot at Benares and we are indebted to Capt. Jennings, R.A.V.C., for his report on the work that was carried out jointly with the author.

EXPERIMENTS

The experiments undertaken were to determine:

- (a) if a satisfactory means of immunizing the goats against pleuropneumonia could be found,

- (b) the earliest point at which immunity became apparent,
- (c) whether strains from different localities were antigenically identical.

Method

Sixty goats were chosen at random from the stock pens. Twenty animals of each type, i.e. large over 70 lb., medium 50 to 70 lb., and small 35 to 50 lb., were taken. The goats were divided into six batches of ten each and each batch contained equal number of the three types of goat. The experimental animals were isolated from the remaining stock and separate attendants provided (this was thought necessary as highly virulent cultures were to be used on these animals). The goats were identified by clipping the batch number on the right flank and the serial number on the left side. Morning and evening temperatures were taken and the reactions to the vaccines noted. The following inoculations were then carried out:

Batch I.—Freshly prepared undiluted pleural exudate and lung mash. The material was obtained from a case of pleuropneumonia which was destroyed in extremis. As far as was possible under field conditions sterile precautions were maintained. Each goat received 0.1 ml. of this vaccine into the ear tip.

Batch II.—The same material as used in Batch I diluted 1/100 with sterile normal saline. Dose 0.1 ml. ear tip.

Batch III.—This vaccine was an attenuated culture of the causal organism obtained from the Indian Veterinary Research Institute. It was a 49th generation culture; 0.25 ml. ear tip.

Batch IV.—This was also an attenuated culture, 85th generation. The same dosage and method of inoculation was used as in III.

Batch V.—A formalinized vaccine prepared on the spot from material obtained from a goat dying of pleuropneumonia. The method of preparation was, briefly, pleural exudate and lung mash, 20 ml. of this added to 10 ml. M/15 phosphate buffer and to this solution formalin was added to a final dilution of 1/1000. This vaccine was stored at room temperature for 24 hours. 5.0 ml. were injected subcutaneously in the neck region. (A difficulty noted here and subsequently was that the pleural exudate coagulated very rapidly and had to be kept cool).

Batch VI.—These 10 goats were kept as controls.

Immunity tests

Goats from each batch including controls were tested out on the twelfth, fourteenth, fifteenth and nineteenth days after inoculation. In addition to these artificially applied immunity tests all experimental goats which became sick and developed pneumonia, etc. were left in the experimental pens thereby ensuring that all the goats were also in contact with diseased animals. It can be claimed, therefore, that immunity tests were fairly vigorous. The animals were tested as follows:

- (a) Twelfth day test, 0.25 ml. lung mash and pleural exudate from freshly dead animal subcutaneously in the neck.

- (b) Fourteenth day test, 0.25 ml. pleural exudate from Izatnagar, stored on ice, subcutaneously in the neck.
- (c) Fifteenth day test, 1.0 ml. of pleural exudate and lung mash from freshly dead goat subcutaneously in the neck.
- (d) Nineteenth day test, 0.5 ml. 3rd generation culture virus from Izatnagar subcutaneously in the neck.

The results of these tests are set out in the Table I.

TABLE I
Results of immunity test.

Batch	Tested on			
	12th	14th	15th	19th day
Control	2/3	1/3	1/1	3/3
Lymph	1/3	0/1	1/3	1/3
Diluted lymph	0/3	1/3	1/1	1/3
Formalinized vaccine	0/3	1/2	0/1	2/4
49th generation culture	0/3	0/3	0/1	1/3
85th generation culture	1/3	0/3	0/1	0/3

Numerator = deaths
Denominator = number of animal tested

The results show that of the control animals 70 per cent died and the remaining goats in this batch were all ill, showing severe local and systematic reactions. In the case of the inoculated animals 23 per cent died. It would appear therefore that immunization of goats against pleuropneumonia would be worthwhile. Of the five vaccines tested, the 49th generation culture virus and the 85th gave the best results; the immunity conferred by the other vaccines, although good, tended to break down when the animals were tested with larger doses of virulent organisms. That a simple vaccine prepared on the spot does give protection is indicated and this method might be worth while adopting when an outbreak occurs. The animals

which resisted natural and artificial infections are regarded as solidly immune. In these immune goats the local reaction following inoculation of virulent material was very slight whereas in the control goats and the goats inoculated with 'lymph' and formalinized juice the local reaction was very severe.

With regard to the time at which immunity became apparent, it would appear from the scanty figures available to be about the twelfth to fourteenth day after inoculation. This will have a bearing on the time required to 'hold' animals prior to transit, since experience in other diseases would indicate that there may be a 'negative phase' in the development of immunity and, to expose newly inoculated animals to the rigours of a train journey may be to invite disaster.

The results also indicate that the strains used at Benares and Izatnagar were antigenically identical; the very slight differences noted are probably due to varying degrees of susceptibility and refractoriness of the goats. The size of the goats did not appear to play any part in the experiments.

Prophylaxis

A. With serum from immunized goats. Two goats for the production of serum were selected from the batch of 15 goats vaccinated subcutaneously in the ear tip with 48th generation culture and later tested for immunity. The history of the two serum donors was, in brief: goat (a), vaccinated ear tip 0.5 c.c. 48th generation culture, no reaction, tested for immunity one month later by insufflation with fresh lymph 'virus', severe temperature reaction for 12 days; again tested for immunity by the same method, three months after the first test, and showed a mild thermal reaction of ten days duration; retested for immunity six months and five days after second immunity test with 0.25 c.c. virulent lymph subcutaneously in the neck and showed local and thermal reaction for five days; finally inoculated subcutaneously in the neck with 0.5 c.c. 4th generation culture three months later, mild thermal reaction three days and bled 200 c.c. fifth day p.i. Goat (b), vaccinated 0.5 c.c. 48th generation culture, very slight local and thermal reaction four to five days, tested for immunity by insufflation with fresh lymph virus ten days after vaccination; slight febrile reaction fourteenth to twentieth day after test dose, retested for immunity five months and twenty-four days after previous test with 0.25 c.c. 13th generation culture given subcutaneously in the neck, decided local and thermal reaction six to seven days after test dose, bled 200 c.c. forty-three days p.i. The subsequent history of these goats after bleeding was uneventful, and both were discontinued two and three months later respectively. The serum was carbolised at 0.5 per cent and mixed and the following tests carried out. Goat 1 was given 0.25 c.c. 4th generation culture subcutaneously in the neck, followed immediately by 10 c.c. serum subcutaneously at the same site. The test was similar in goat 2 the serum being given six hours after the dose of culture. Goat 3 was kept as control, being given 0.25 c.c. 4th generation culture subcutaneously in the neck. Both, serumized goats reacted severely and died of pleuropneumonia; the control reacted badly and survived. The test was repeated in three more goats and all three reacted severely and died of pleuropneumonia. In a third test, three goats were insufflated with 2 c.c. 4th generation culture and two were given 10 c.c. serum subcutaneously in the neck, one at the height of the temperature reaction (sixth day) and the other

at the beginning of the febrile reaction (second day). Both these goats and the third goat used as control reacted severely and died of pleuropneumonia.

B. *With novarsenobillon*.—*Gudcheft [1928], quoted by Curasson [1936] used salvarsan with good results at a dose rate of 0.2 to 0.7 gm. according to body weight and claimed 88.4 per cent of cures in 746 animals treated. Novarsenobillon was used in the treatment of contagious pleuropneumonia of the goat by *Crawford (personal communication) in Ceylon, in 1932. Good results were claimed in the control of the disease 'provided that it was of the epizootic and not of the sporadic type'. In one outbreak, for example, nine goats had died within a few days with typical post-mortem lesions. Twenty-seven other cases were treated with novarsenobillon and recovered. Even advanced cases which seemed beyond hope of recovery yielded to treatment and fifteen of the twenty-seven goats treated were sold for slaughter in excellent condition about a year later. Encapsulated lesions in the lungs, similar to those seen in 'lungers' in contagious bovine pleuropneumonia, were found in a certain number of these goats. The drug was given intravenously at a dose rate of 0.45 to 0.8 gm. according to the size of the goat. One dose was usually sufficient but had cases were given a second dose after three days. *Gopalkrishnan (personal communication) reported favourably on this treatment in cases of the disease in Assam, and recommended a dose rate of 0.15 to 0.30 gm. depending on the size of the goat. The cases selected for treatment were in the more early stage of the disease; fever commenced to abate twenty-four hours or so after the administration of the drug, and general improvement was seen within two—three days. In cases which exacerbated, a second injection of the drug at the same dose rate was indicated four to five days after the first injection.

Experimental treatment of contagious pleuropneumonia of the goat with novarsenobillon (May and Baker) was carried out in goats at Izatnagar; the drug was fresh stock and used strictly according to makers' instructions. Small sized goats were used in these tests and the dose of the drug was accordingly estimated. Goats 1 and 2 were inoculated subcutaneously in the neck with 2 c.c. lymph 'virus' stored for seventy days at 5°C. A moderate local reaction ensued in goat 1, followed by fever and signs of pneumonia twenty-two days p.i., ending in recovery forty-three days p.i. Seventeen days later, this goat was insufflated with fresh lymph and reacted severely; 0.225 gm. novarsenobillon was given intravenously at the height of reaction, i.e. fifth day, and as there was no improvement by the ninth day a second dose of 0.45 gm. was given. The drug had no apparent effect on the course of the disease and death occurred on the twelfth day with typical lesions of pleuropneumonia. Some toxic effects followed the second dose, viz. profuse salivation, muscle tremors and some signs of collapse, but these were fleeting. The history of goat 2 was similar, except that it showed only a mild local and thermal reaction in response to the inoculation of refrigerated virus, it was insufflated along with the previous goat and given 0.225 gm. novarsenobillon at the height of fever at the sixth day, and 0.45 gm. at the tenth day, with the same results as in goat 1. The test was repeated in three more goats inoculated subcutaneously in the neck with 1 c.c. fresh lymph 'virus'. All three goats reacted severely, and each was given 0.225 gm. novarsenobillon intravenously at the peak of the reaction. This was entirely without effect, the reaction progressing to death with lesions of pleuropneumonia in

each case and if anything, the drug seemed to accelerate the fatal outcome. Finally, three goats were subcutaneously inoculated in the neck each with 3 c.c. lymph 'virus' and, twenty-four hours later, two were given 0.3 c.c. novarsenobillon intravenously, the third goat serving as control. The treated goats reacted as severely as the control, death supervening six, nine and five days p.i. respectively, with specific lesions of pleuropneumonia.

DISCUSSION

There is little doubt, on the evidence available in the literature, that epizootic pleuropneumonia of the goat in India is the same disease as has occasioned much fruitful research on the part of workers in Africa, Italy, Greece, France and elsewhere. Mettam* [1929] and Walker* [1932] showed that the disease could be transmitted in healthy goats with filtrates of pleural exudate from clinical cases of the disease, and Kearney* [1927] obtained a suspicious opalescence suggestive of growth of a pleuropneumonia organism in tubes of Martin's broth sown with heart blood, spleen and pleural exudate. Oliver* [1916], Taylor* [1920], Edwards* [1923 to 1926] and Longley [1940] in India and *Hutcheon [1889] in Africa and others considered that, on pathological grounds, the disease resembled contagious bovine pleuropneumonia. Longley [1940] in India, *Hutcheon and Henderson [1929] in Africa and *Mori [1918] and *Crimi [1926] in Italy achieved a definite measure of success in the control of the disease by vaccination along lines similar to those practised in the control of contagious bovine pleuropneumonia. The work done in recent years on contagious pleuropneumonia of the goat in India is largely a restatement of the pioneering research referred to, with emphasis on the isolation of the causal organism and on immunity developed along modern lines accepted in the control of the analogous disease in bovines. In the investigation into outbreaks of pleuropneumonia of the goat in India during the last five years, a filtrable organism of the pleuropneumonia group has been shown to be the aetiological factor. The disease caused by this organism has a wide distribution throughout India and is, with the possible exception of the disease due to nematode parasites, the major disease of goats in India. The experimental work undertaken shows the highly infectious nature of this disease. Contrary to the observations of Longley [1940], the disease spreads rapidly in healthy goats mixed with diseased goats, even when there is no direct contact; infection could be transmitted at a distance of not less than twenty feet in a shed. Further, it has been shown that sheds are infective for at least one month after the last fatal case. These experiments indicated the ease and rapidity of an air-borne infection as well as the tenacity of the organism under field conditions. When lymph virus (pleural exudate) from a field case was first tested in goats at Izatnagar, the preliminary results did not indicate a high order of virulence of the material, whether insufflated in goats or given subcutaneously in the neck. After a few animal passages, however, the virus was exalted in virulence for the type of animal used, so that the results of inoculation attained a reasonable uniformity. At this stage, and in subsequent experimental work, forty of forty-seven goats insufflated with the virus reacted and died of specific pleuropneumonia. The results were even better following subcutaneous inoculation of a few drops of the 'virus' in the ear tip of goats, all of twenty-two goats so inoculated showing a specific,

fatal reaction with pleuropneumonia on autopsy. It was, therefore, a matter of choice whether fresh virus for experimental work was obtained by one or the other method of inoculation. The lymph 'virus' so obtained was fully virulent when tested in healthy goats by subcutaneous inoculation in the neck, after three months and somewhat attenuated after three and a half months' storage at 5°C. In this respect, the virus of goat pneumonia resembles the virus of contagious bovine pleuropneumonia. In contrast, Longley [1940] described the strain of virus responsible for pleuropneumonia of the goat in Madras Province as being unusually fragile, and non-infective after 96 hours at 0 to +5°C. A second point of difference worth noting is that Longley described both the natural and the experimental disease as essentially non-febrile, which is contrary to our experience. One point of agreement is that the Indian sheep is susceptible to subcutaneous inoculation in the neck with the Madras and Bombay strains of the virus (other strains not tested). Seven of eight ewes tested by Longley [1940] with the Madras strain reacted fatally with full local reactions, but the same 'virus' was without effect when insufflated in healthy sheep. Of seven sheep tested at Izatnagar with the Bombay strain, three reacted severely with fever and intense progressive oedema at the site of inoculation and with acute pulmonary congestion on autopsy. Intense exudative peritonitis and pericarditis were seen in the fourth sheep and two of five sheep insufflated with pericardial exudate from this case reacted, one fatally, with lesions of exudative peritonitis and pleuropneumonia similar in type to that seen in goats insufflated with lymph virus. It is clear, therefore, that the sheep is relatively susceptible to the virus of pleuropneumonia of the goat, and it is not beyond the bounds of possibility that cases of this disease do occur in sheep in the field, although these have not been reported. The symptoms of the disease are definite and no difficulty should be experienced in the diagnosis of clinical cases; the affected animal's stance, respiratory distress, cough, nasal discharge and high fever are cardinal signs. The lesions of the disease afford the field worker reasonable grounds for a diagnosis of pleuropneumonia due to *Borrelomyces peripneumoniae capri*. The nature of the lesions must be assessed on a sufficient number of autopsies, and the analysis of the aggregate must conform to the following pattern: lesions of pleuropneumonia tend to be unilateral, the anterior and middle lobes most commonly involved. Pleurisy is fibrinous in type and early organization with firm adhesions to the chest wall and often to the pericardium are seen; pleurisy is often limited to the affected area of the lung, or it may be patchy; pneumonia may be bilateral and pleurisy unilateral, or vice versa. Haemorrhagic infarcts anywhere on the lung surface, even when the surrounding lung area is not pneumonic, thrombosis of branches of the pulmonary artery, interstitial oedema or frank marbling of the lung, a varying amount of a straw coloured, rapidly clotting exudate in the chest cavity or within the pericardium are all lesions of great significance. In addition, the histological changes in the affected lung tissue in various stages of development of the pneumonic process are quite specific. Finally, the subcutaneous inoculation in the neck or intramuscularly of a few healthy goats with 2-3 c.c. pleural exudate or saline lung mash will provoke, in one or more goats, a specific syndrome and lesions at the site of inoculation and within the underlying muscle, and frequently pleuropneumonia. No information is available from the field to show whether recovery from the disease protects against second attack. The experimental data now recorded shows that some measure of immunity is gained by goats that survive

the parenteral inoculation of the virus subcutaneously in the neck. Thus, of six survivors which reacted severely to the inoculation of lymph 'virus' four were solidly immune to test insufflation with fresh homologous lymph which provoked a fatal reaction with pleuropneumonia on autopsy in six controls. The complete lack of immunity in two goats which reacted as badly as the controls cannot be explained. It is just possible that the interval between the original inoculation and the immunity test was too long in the case of the non-immunes; these were tested at fifty-two and sixty-one days after the first inoculation as compared with eighteen, twenty-eight and forty-two days, although the fourth immune was the pair of two goats tested at fifty-two days. It must also be considered whether the test employed was not too drastic in comparison with a natural field infection. The results obtained by Longley [1940] in Madras goats with a formalinized lymph virus vaccine could not be confirmed in goats vaccinated by this method at Izatnagar with a vaccine prepared from the Bombay strain according to Longley's formula and subsequently tested for immunity by insufflation with the homologous strain.

The question of the susceptibility ratio of goat populations in different parts of India is obviously of great importance in the general bearings of immunity and particularly in the application of the methods to be applied, once the principle is accepted that more than one method of vaccination is available which, on reasonable test, is proved capable of conferring a useful immunity when properly applied. A method of proved value in one province might prove positively dangerous in another province. Thus, Longley [1940] showed that a satisfactory level of immunity was obtained in goats vaccinated subcutaneously in the ear tip with fresh lymph 'virus'. The size of the dose recommended (5 c.c.) indicated a relatively low order of susceptibility in the goats so vaccinated. In comparison with this result, a relatively small dose of fresh lymph virus (0.25 c.c. Bombay strain) uniformly caused fatal reactions, with pleuropneumonia, when given by the same route in goats at Izatnagar, and the same results ensued even when the virus was rubbed in tightly on the scarified ear. Nothing is known of the ratio of susceptibility to the local virus of different breeds of goats or of goat populations in different areas in India, but this is a factor which would have to be reckoned with in using live vaccines. It might be argued that the method of vaccination with the highest level of safety compatible with apparent satisfactory reduction of disease incidence in the field is the requisite criterion, and that such a vaccine could be universally applied. However, we would always prefer to advocate the use of a really potent vaccine just within the limit of safety. This opinion is in accordance with the accepted ideas in the control of the analogous disease, in which it has been shown that there is a quantitative ratio between virulence (or attenuation) of the living organism vaccine and the antigenic response in vaccinated animals. If goats in an area can generally withstand vaccination with fully virulent lymph virus, as has been shown by Longley [1940] in Madras, then this is obviously the method to employ the material for vaccination is always easily obtained either as pleural exudate from a field case or from a donor goat insufflated or subcutaneously inoculated with the same material. The method is simple and direct and can be employed even by stockmen. The decision to employ the method would depend on the results of a preliminary test with lymph virus of a reasonable number of goats in the area selected for wholesale vaccination. This method was put to a field test in a village in the North-West Frontier Province where the disease

had been smouldering for some months with serious losses. The goat population at the time of visit was about six hundred and three hundred and twenty-one goats were vaccinated subcutaneously in the ear tip with 0.5 to 1.0 c.c. lymph virus obtained from a clinical case destroyed in extremis; the remaining goats were left unvaccinated as controls. Vaccination was reactionless in all of the three hundred and twenty-one goats. Nineteen of those goats died of pleuropneumonia within seventeen days of vaccination, and thereafter, no fresh cases or losses occurred in this batch. Seventeen of the nineteen goats died two to seven days after vaccination and the presumption is that the majority of these animals were already in the incubation phase of the disease, if not actually affected, at the time of vaccination. It was apparent that vaccination had cut short the incidence of the disease which continued unabated in the unvaccinated lot, in which the mortality was 75 per cent as compared with three per cent in the vaccinated lot; this figure allows for losses in goats vaccinated in the incubative stage of the disease. These results compare favourable with those recorded by Longley [1940] in Madras where the mortality rate in goats so vaccinated was 5.4 per cent compared with 75 per cent in unvaccinated controls. We also agree with Longley's observation that some losses in goats during the first two weeks after vaccination in the face of an outbreak must be expected as immunity is somewhat slow in building up and is not fully developed until about fourteen days after vaccination.

Experiments in highly susceptible goats at Izatnagar show that Bennett's broth cultures of the goat pleuropneumonia organism from the 43rd to 80th generation (later generations not tested) are, on the whole, well tolerated when given in moderate dosage subcutaneously in the ear or the tail tip. Thus, of ten goats vaccinated with 43rd generation culture, five ear tip and five tail tip, one goat inoculated ear tip died of pleuropneumonia fifty-two days p.i. after a delayed reaction, the remainder showing no reaction. Five goats died of intercurrent disease before test and the four survivors were solidly immune on test with homologous lymph 'virus' given subcutaneously in the neck, four controls reacting fatally and dying of pleuropneumonia. Of fifteen goats vaccinated ear tip with 48th generation culture a fleeting reaction was seen in one goat; four were tested for immunity with lymph 'virus' given subcutaneously in the neck sixteen days p.i. and three reacted and died of pleuropneumonia; of four goats tested in the same way twenty-eight days p.i. two reacted fatally and one slightly; of seven tested by insufflation thirty-one days p.i. five were solidly immune and two reacted fatally. Of ten goats vaccinated ear tip with 71st to 74th generation culture none reacted and all were immune when tested twenty to twenty-five days p.i. with 8th generation culture given subcutaneously in the neck, both controls reacting fatally. Of ten goats vaccinated ear tip with 80th generation culture, severe fatal reaction was seen in two goats, and a mild reaction with quick recovery in four goats. The eight survivors were solidly immune thirty-seven days p.i. to test with 15th generation culture given subcutaneously in the neck the control reacting fatally. Thus, of forty-five goats vaccinated with culture between the 43rd to 80th generation, eight goats reacted to vaccination, three cases terminating fatally (6.6 per cent), five died of intercurrent disease and, of the thirty-seven survivors subsequently tested for immunity, thirty proved immune (81 per cent). The majority of these fatal issues occurred in goats tested with highly virulent lymph 'virus' given subcutaneously, five of twelve goats so tested dying

of pleuropneumonia (41.6 per cent) as against two of twenty-five (8 per cent) tested with 8th to 15th generation culture. In the twelve goats tested for immunity with lymph 'virus' all deaths occurred in the group tested sixteen to twenty-three days after vaccination, and none in the group tested at thirty-nine to fifty-one days. It is possible that this observation has some significance. The results of the test of the 45th generation culture in an infected batch of goats in the North-West Frontier Province were comparable with the results obtained under much the same conditions in that province with fresh lymph 'virus' as a vaccine. It would appear from the results of the immunity tests in general that measures now exist which can do much to mitigate the ravages of contagious pleuropneumonia of the goat in India, and the work of Longley [1940] in this connection is largely confirmed.

CONCLUSIONS

A survey of the literature indicates that contagious pleuropneumonia of the goat in India is the same disease that occurs, with equally devastating losses, in many parts of Africa, in Italy, Greece, France and other countries of Europe, in Asia Minor and probably in Ceylon.

In India, proof is adduced that this specific disease of the goat exists in the Provinces of Madras, Assam, Bombay, Sind, the North-West Frontier Province and in the Central Provinces and the United Provinces.

The symptoms, course and pathology of the disease are described in detail.

The disease spreads rapidly by direct contact between diseased and healthy goats and by air-borne infection in a shed at a distance of not less than twenty feet. Infected sheds remain infective for at least one month after the last fatal case.

The disease can be transmitted to susceptible goats by insullation with pleural exudate from natural case of the disease, and the virus be thus maintained indefinitely in serial passage. Subcutaneous inoculation in susceptible goats of the same material causes a specific local lesion, and frequently specific pleuropneumonia. Cattle and small laboratory animals are refractory to this inoculation and sheep somewhat susceptible, although natural cases of the disease have not been detected in sheep in the field.

Lymph 'virus' (pleural exudate) retains full virulence for goats when stored for three months at $+5^{\circ}\text{C}$. and shows decided loss in virulence at three and one half months.

The infective agent in lymph 'virus' passes Seitz E.K. and Berkfeld V filters at moderate negative pressure. Such filtrates are as pathogenic in susceptible goats as fresh lymph 'virus'.

The causal organism may be readily isolated from such filtrates sown in tubes of Bennett's broth or agar with ten per cent healthy goat serum added.

Such cultures reproduce the disease in susceptible goats. The causal organism conforms in morphological and cultural characters to the organism causing contagious Bovine pleuropneumonia and the name *Borrelomycetes peripneumoniae capri* seems appropriate in the designation of the causal organism of contagious pleuropneumonia of the goat.

The organism undergoes definite attenuation in virulence when subcultured at seven to ten day intervals in the medium described. With the Bombay strain attenuation was apparent at the 43rd generation culture.

Suitably attenuated cultures may be safely used in the vaccination of susceptible goats.

Formalized lymph 'virus' is of little, if any, value as a vaccine.

Serum from goats immunised with attenuated culture and later fortified with virulent lymph 'virus' was found to have no protective value when given subcutaneously in goats at various stages of the experimental disease. The same result was observed even when serum and virus were given simultaneously, or within a few hours, on opposite sides of the neck of susceptible goats.

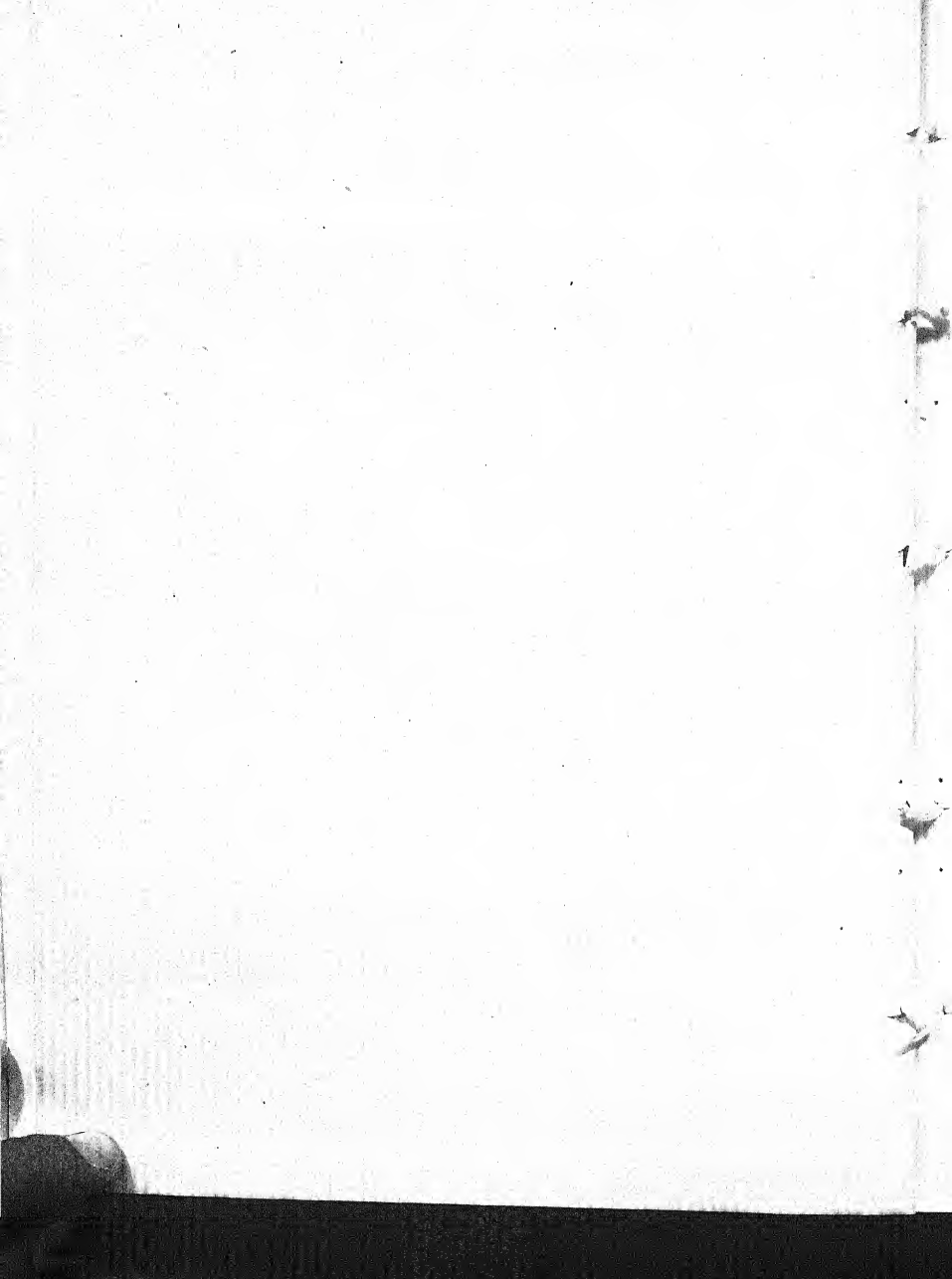
Novarsenobillon (May and Baker) had no prophylactic value in experimentally infected goats treated at various stages of the disease. The drug had no controlling effect on the initiation of the disease syndrome when given simultaneously with the virus.

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Captain Jennings, R. A. V. C., served for a short period as Liaison Officer between Army Headquarters, India, and the Indian Veterinary Research Institute, being detailed to investigate the possibilities of vaccination of army goats in the field. This officer's valuable contribution to the investigation was highly appreciated.

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STAPHYLOCOCCUS INFECTION IN CHICKS

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(With VI Plate)

IT has been observed that chicks undergoing immunization against Ranikhet disease may occasionally develop symptoms of incoordination and lameness. Haddow and Idnani [1946] observed that the proportion of birds going lame after vaccination against Ranikhet disease was about two per cent. Since, on more than one occasion, the number of birds going lame after the vaccination exceeded this percentage further investigation was undertaken on this problem, with a view to elucidating the aetiology of the condition.

Jungherr [1933] showed that an organism of the characters of *Staphylococcus aureus* was responsible for arthritis of the tibio-metatarsal joints in cases of lameness in seven month old turkeys. The causal organism was isolated from the heart blood in acute cases and from the affected joints in chronic cases. Plastringe and Jungherr [1940] made observations on the pathogenicity of an avian strain of *staphylococcus* isolated from the joints and the skin of natural or induced fowl-pox lesions. Jungherr and Plastringe [1941] investigated cases of arthritis of the tarsometatarsal joint of five month old turkeys, caused by a *staphylococcus*. The organism coagulated rabbit plasma and haemolysed ox and rabbit blood. It was considered that the insertion of 'Spees' or 'pick-guards' through the nostrils was a contributory factor in the spread of the disease.

OBSERVATIONS

Four cases of lameness occurred in a batch of twenty chicks, two to four weeks after vaccination at six weeks of age. Progressive lameness was observed in the affected birds, which were destroyed. Post-mortem examination showed no macroscopic lesion in any of the internal organs. Cultures of a Gram positive micro-organism, occurring in pairs and clusters, were, however, obtained from the heart blood, liver, tibio-metatarsal joints and bone marrow. On another occasion similar cases of lameness were observed among known healthy chicks of about three months of age and such affected birds died in the course of about a week. On that occasion, also, none of the internal organs showed any gross lesion and a similar organism was isolated from the heart blood and the joints. The occurrence of this manifestation of lameness combined with the isolation of *staphylococcus* ruled out the possibility of viewing the lameness, among the vaccinated birds, as one of the effects of the vaccination, and the observation was, subsequently, confirmed by similar findings in healthy and vaccinated birds.

Symptoms

The affected chicks evinced a locomotor incoordination rapidly worsening along with decided malaise, anorexia and ruffled feathers, disinclination to move and marked lameness on forced movement. Examination of the legs showed a slight swelling of one or both tibio-metatarsal joints. The lameness was generally unilateral though in a few cases both the joints were affected. Some of the cases improved, whereas in others the condition deteriorated with in-turning of the toes, (Plate VI). That phase took about one to two weeks to develop and continued for several weeks, a few of the birds succumbing to the condition. From such cases the organism could be isolated only from the affected joints. On post-mortem examination none of the internal organs exhibited any naked eye lesion. The affected tibio-metatarsal joints, however, showed a slight swelling and haemorrhagic spots over the articular surfaces. In a few cases, particularly in two to three week old chicks, the affection was acute, such birds dying within two days. In those cases the organism was isolated from the heart blood, bone marrow and the joints.

Bacteriology

Bacteriological examination showed an organism in pure culture with the following characters: Gram positive cocci arranged in pairs, tetrads or in grape like clusters, growing well on plain agar and blood agar, the colonies being white, shining, round and convex. On blood agar plates no haemolysis was observed. The organism liquified gelatine. Acid was produced in dextrose, lactose, sucrose and mannite, with no change in raffinose, salicin and inulin. M. R. and V. P. reactions were negative and there was no reduction of nitrates and neither indol nor ammonia was produced. The organism was H_2S positive, litmus milk was clotted with the production of acid and rabbit plasma was not coagulated.

The organism was identified according to Bergey [1939] as belonging to *Staphylococcus* s.p. and probably *Staphylococcus albus*. The organism was compared with a known strain of *S. albus* available at this Institute and was found to show identical cultural characters and biochemical reactions.

Pathogenicity

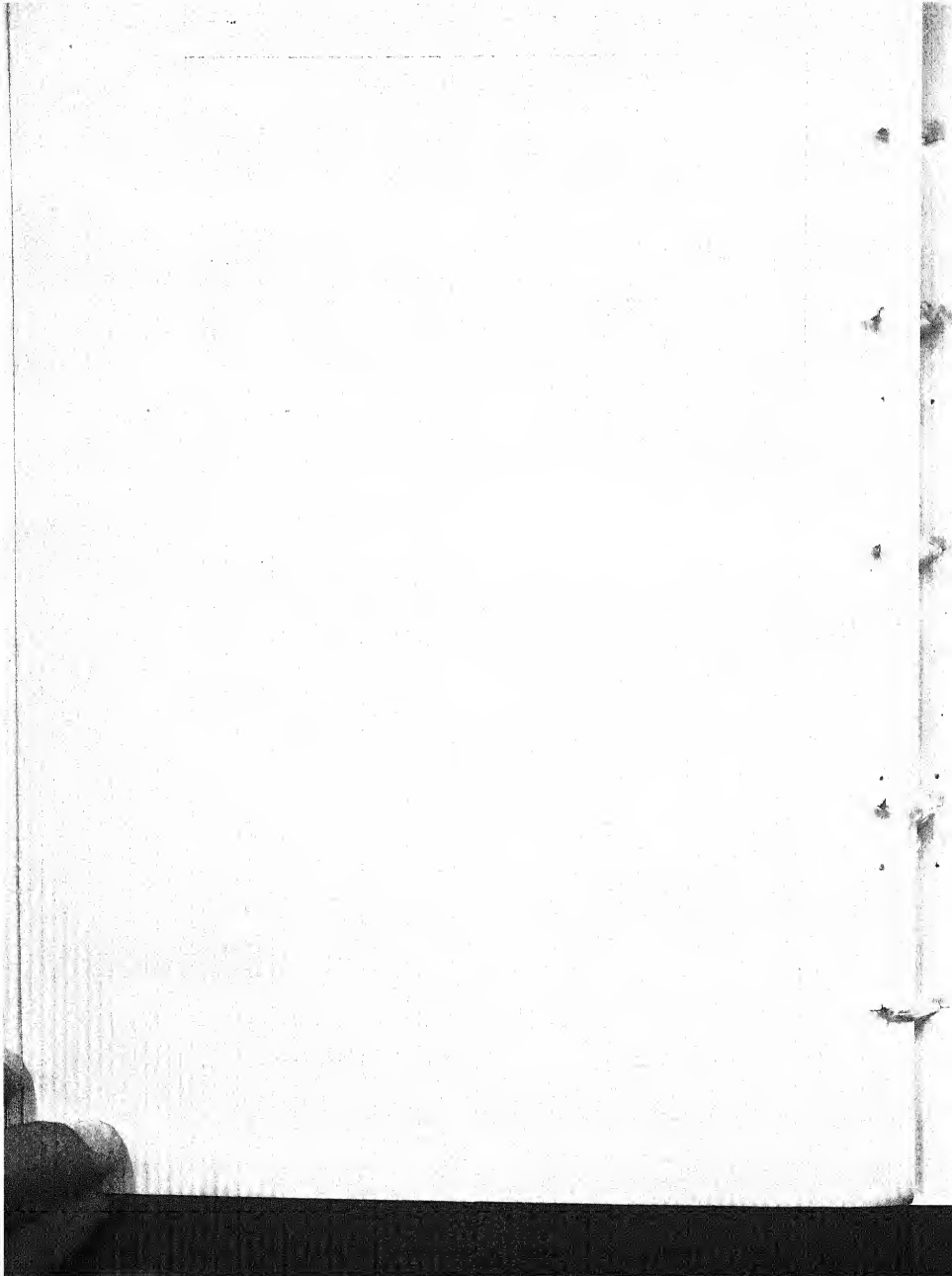
Subcutaneous inoculation of the organism, in rabbits and guinea-pigs, produced acute inflammation at the site of inoculation, generally accompanied by thermal reaction and dullness and lasting for two to four days. Intravenous injection in adult birds of 0.5 c.c. of broth culture, incubated for 24 hours at $37^{\circ}C$., produced in a majority of cases a chronic infection but in chicks two to four week old an acute infection was set up. Subcutaneous inoculation set up a similar generalized infection accompanied by acute inflammatory changes at the site of inoculation. Feeding of the culture in the form of a suspension in drinking water or well mixed with the grain ration did not produce the disease.

DISCUSSION

Reference to the literature cited indicates that pathogenic *staphylococci* are generally coagulase positive and haemolytic. But in this article is recorded the



Affected chicks showing further deterioration with the in-turning of toes



occurrence of an infection of chickens due to a *staphylococcus* which is atypical in that cultures were coagulase negative and non-haemolytic. But the pathogenicity test proved conclusively that the organism isolated from the lesions described was the precise aetiological factor concerned. It is generally recognized that a certain percentage of birds go lame for a fairly long period as the result of vaccination against Ranikhet disease, but only 0.5 per cent of vaccinated birds may be permanently maimed. On occasions where the percentage of cases of lameness is considerably higher, an intercurrent infection of the type described in this paper may be suspected. It is difficult to account for such an infection in a minority of vaccinated birds, but it is possible that one effect of the introduction of the Ranikhet disease virus into chickens is a stimulation of an otherwise latent or commensal infection with the strain of *S. albus*.

SUMMARY

Lameness in birds, caused by a Gram positive, non-haemolytic but pathogenic *staphylococcus* is described.

Subcutaneous inoculation in rabbits and guinea-pigs produced acute inflammation at the site of inoculation accompanied by thermal reaction. Intravenous inoculation of the organism in adult fowls and young chicks produced chronic and acute infections respectively.

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RINDERPEST IN WILD RUMINANTS

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(With two text—figures)

SUSCEPTIBILITY of wild game to rinderpest has been known for a long time [Poulton, 1927 ; Carmichael, 1933] and outbreaks have been recorded in countries like Africa in which the disease spreads to domestic animals Thomas and Reid, 1944]. Du Toit [1947] has suggested that the presence of game is largely responsible for the continuance of rinderpest in East and West Africa in spite of wide-spread vaccination of the cattle. The possibility of the disease occurring in wild animals in India has been suspected but the reported information is limited to rare records of natural outbreaks in zoo animals [Mohan, 1944]. This paper records another such outbreak which occurred at the Zoological Gardens, Lucknow, during the winter of 1946.

HISTORY OF THE OUTBREAK

As far as could be ascertained, that was the first outbreak of rinderpest that occurred in these gardens.

Before the outbreak, the paddocks concerned were occupied by the following animals :

Paddock	Animals	Number
A.	1. Sambhar deer (<i>Cervus aristotelis</i>)	21
	2. Hog deer (<i>Cervus porcinus</i>)	12
	3. Black buck (<i>Antelope cervicapara</i>)	2
B.	1. Spotted deer (<i>Cervus axis</i>)	69
	2. Four-horned antelope (<i>Petruceros quadricornis</i>)	4
	3. Black buck	1
C.	Barking deer (<i>Cervulus muntjac</i>)	14
D.	Neelgai (<i>Boselaphus tragocamelus</i>)	2
E.	Neelgai	2
F.	Nil.
—		127

The location of the affected paddocks and the surrounding area is shown in the sketch below (Fig. 1). The dates on which the disease broke out in the different paddocks are also given.

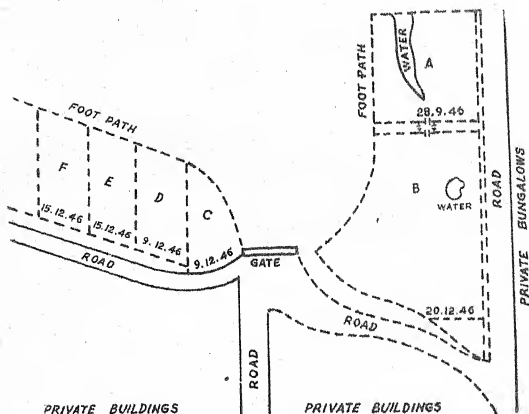


FIG. 1. Location of the affected paddocks

Two sambhar deer in paddock 'A' which passed dysenteric stools for a day or so, were found dead on the 28th September 1946. During the subsequent 10 days, two hog deer and two more sambhar deer died after showing similar symptoms. Further deaths in this paddock did not occur until the 17th November, but on the 20th October the disease appeared in paddock 'B', in which one or two spotted deer began to die occasionally after showing approximately the same set of symptoms as noticed in the sambhar deer. Subsequent increase in death rate to two or three a day gave rise to the suspicion of contagious disease. From the 17th November to the 4th December the disease was in full swing in paddocks 'A' and 'B' after which, leaving only four hog deer which survived the outbreak in the former paddock and eight spotted deer in the latter, it appeared simultaneously in paddocks 'C' and 'D' on the 9th December, and later (on the 15th December) in paddock 'E'. After the death of one barking deer on paddock 'C' (on the 9th December), the remaining animals of this paddock were transferred to paddock 'F' which was vacant. Deaths, however, continued to occur in this last paddock, six barking deer dying there. The last death occurred on the 31st December.

ORIGIN AND SPREAD OF THE DISEASE

All possible efforts were made to keep the wild animals in their natural environments and they were in good health before the outbreak. Each paddock provided a wide area for grazing, which was supplemented by grass from outside. Paddocks 'A' and 'B' had artificial ponds for drinking water supplied from a common tap

located in paddock 'A'. Each animal was in the habit of coming to receive its share of concentrate (whole gram) distributed every morning and any one lagging behind was usually found to be sick.

In the absence of any chances of direct contact between the zoo animals and the outside animals, the factors considered possibly responsible for the origin of the disease were (i) grass, which was purchased mainly from outside, and (ii) visitors, who often patted and offered eatables to these animals. There was, however, no direct evidence to definitely incriminate any such factors and it was quite possible that the outbreak was the result of air-borne infection. Two cows in a neighbouring bungalow, opposite paddock 'A' died of rinderpest in the third week of September. Cattle in two nearby villages were also affected with rinderpest about the same time. The unenclosed grounds of the Gardens were open for grazing to outside animals.

The inter-paddock spread of the disease might again have been due to (i) air borne infection, (ii) common attendants, and (iii) grass which was supplied from the same source to all the paddocks. The common source of water in the case of paddocks 'A' and 'B' might have been responsible for the spread of infection from the former paddock to the latter.

Mortality in different species

The figures of mortality are given below in Table I. The species concerned are listed in descending order of percentage of deaths in them. This would probably also represent the order of susceptibility.

TABLE I
Figures of mortality

Species of animals	Total number	Deaths	Percentage mortality
1. Sambhar deer	21	21	100.0
2. Black buck	3	3	100.0
3. Four-horned antelope	4	4	100.0
4. Spotted deer	69	61	88.4
5. Neelgai	4	3	75.0
6. Hog deer	12	8	66.6
7. Barking deer	14	7	50.0

According to Poulton (loc. cit.) several species of game in Uganda, viz. buffalo giraffe, eland, kob, bushbuck, sitatunga, reedbuck, giant hog, pig, and wart-hog are susceptible to rinderpest. Blue wildebeeste and koodoo have also been known to suffer. In the outbreak recorded by Mohan (loc. cit.) in the Zoological Gardens, Alipore, Calcutta, the animals affected are said to have been deer, gayals, etc.

Symptoms

Dullness, tendency to remain aloof from the rest of the herd, disinclination to receive the share of concentrate, and shooting bloody diarrhoea were constant features in all the sick animals. Buccal mucosa, including tongue of one spotted deer which could be secured with great difficulty, was covered with easily removable greyish deposits. Death usually occurred in a day or two after the onset of symptoms. The weekly mortality is shown in the following graph dating from the first death (Fig. 2).

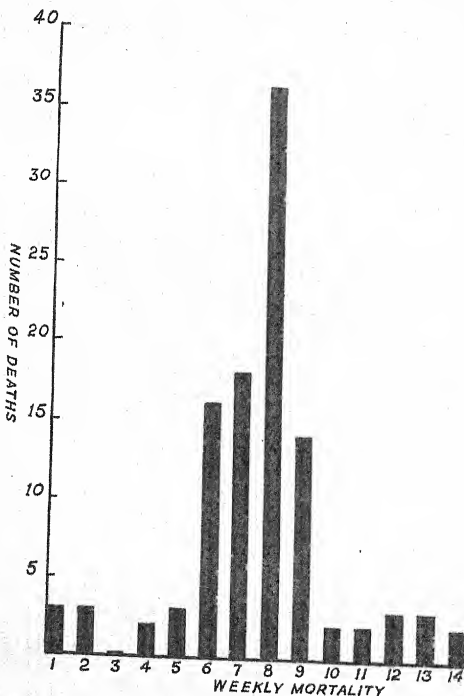


Fig. 2.

Post-mortem features

In the nearly two dozen carcasses which were autopsied, hind quarters were soiled with bloody diarrhoea. The buccal mucosa was covered with greyish deposits, which could be easily removed leaving fine raw ulcers. The oesophageal mucosa was similarly affected in several cases. The mucosa of the abomasum was congested and ulcers on its folds in the fundus were constant lesions. The small intestines contained dark brown liquid ingesta mixed with blood and mucus shreds; the mucosa was highly congested, swollen and covered with greyish deposits. Congestion and ulceration were also present in the large intestines in varying degrees. The upper air passages were highly congested and occasionally showed petechial haemorrhages. The lungs were generally in a state of active congestion and showed areas of consolidation in some cases. There were occasional sub-endocardial haemorrhages.

Diagnosis

Blood films always proved negative and cultures from heart blood and viscera made in a few cases revealed no pathogenic organism.

The disease could not be transmitted to rabbits and guinea pigs by subcutaneous and intraperitoneal injections of saline suspensions of liver and spleen collected from fresh carcasses of sambhar deer, but the two goats inoculated subcutaneously with spleen suspension showed thermal reaction from the third day. One of these was destroyed on the fifth day and the infection was reproduced into another goat through subcutaneous inoculation of the spleen suspension. The second goat showed thermal and other reactions suggestive of rinderpest but survived. When tested against virulent rinderpest goat tissue virus, it proved to be immune. Another goat, injected with Seitz-filtered spleen suspension, showed a reaction similar to that seen on the goats injected with unfiltered material. Material collected from the carcass of a sambhar deer also proved positive for rinderpest when tested at the Indian Veterinary Research Institute, Mukteswar.

The histo-pathological examination of the material (lung, liver, spleen and rectum) forwarded to the Indian Veterinary Research Institute, Mukteswar, did not reveal anything significant, except in the rectal mucosa which was petechiated and showed much desquamation and erosion of the epithelium.

The highly suggestive clinical symptoms and post-mortem lesions, contagious nature of the disease, survival of the only animal inoculated with anti-rinderpest serum and the history of rinderpest outbreak in the adjoining locality led to the provisional diagnosis of rinderpest. Successful transmission of the disease to goats, particularly with Seitz-filtered material, and the immunity of the tested goat to subsequent challenge with virulent rinderpest virus confirmed the diagnosis.

Control measures

Owing to the semi-wild conditions under which these animals were maintained and consequently to their intractable nature, it did not become possible to adopt vigorous measures like inoculation of anti-rinderpest serum, except in the case of one spotted deer which was secured after considerable struggle. This animal

survived the outbreak. The infected places were disinfected with lime and the excreta was burnt. The water pond in paddock 'B' was fenced off and fresh drinking water was supplied from a tube-well. A little quantity of potassium permanganate was added to the drinking water and the watering vessels were also frequently washed. Separate attendants were employed for infected paddocks and carcasses were buried deep with lime.

SUMMARY

Record is made of an outbreak of rinderpest in the Zoological Gardens, Lucknow.

The diagnosis was confirmed on animal transmission and immunity tests.

The clinical and post-mortem features are described and account is given of the mortality occurring in the different species.

ACKNOWLEDGEMENTS

The histo-pathological examination and some biological tests were done at the Indian Veterinary Research Institute, Mukteswar. The assistance of Shri J. L. Govil of the Biological Products Section, Lucknow, is gratefully acknowledged. The authors are indebted to Shri R. N. Mohan, Veterinary Investigation Officer, U. P., for correcting and recasting this paper.

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A NOTE ON A VARIANT OF *PASTEURELLA AVISEPTICA*

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(Received for publication on 4 July 1949)

THE existence of various types of *Pasteurella* differing from one another in their type characters has already been recognized by workers abroad who have classified them using various methods for identification. Jones [1921] classified according to fermentation reactions, Cornelius [1929] differentiated them by agglutination. Many others have adopted precipitation, motility or cross immunity, etc. as criterion for classification. Roberts [1947] proved by immunological methods the existence of different strains of *Pasteurella septica*. Their natural classification according to animal susceptibility as *Pasteurella bovisseptica*, *Past. suisseptica* and *Past. avisseptica*, etc. does not seem to be satisfactory as, according to Roberts, strains of the same type exist behaving differently in their biochemical reactions; similarly strains from different species of animals come under the same type immunologically although one is virulent to certain species of animals and not to others. Thus, in his type (I) he has included both *bovine* and *porcine* strains and in type (II) *avian*, *bovine* and *porcine*. A single *avian* strain from India which he examined was found to be a type of its own which at the same time differed from an Australian *avian* strain. Iyer [1944] also reported the existence of more than two serological groups amongst the strains of *Past. avisseptica* he examined. It is not definitely established whether such differential behaviour is an acquired character due to environment although Iyer claims success in transforming a strain of *bovisseptica* into a virulent strain for fowls after passing through pigeons successively; but he has not definitely established the type characters of such strains.

During the course of an examination of a culture of *Past. avisseptica*, we found the organism behaving in a different way in its biochemical reactions from the standard type strain of *Past. avisseptica* maintained in the Institute. Two culture tubes of organisms isolated from ducks, from an out break reported to be of fowl cholera among ducks, were received from the Assistant Disease Investigation Officer, Madras for typing, with a report that a 24-hour broth culture in 0.5 c.c. doses killed a rabbit, pigeon and duck in 12, 24 and 48 hours respectively but failed to kill a fowl. Sub-cultures were made from the original tubes. The sub-culture along with a culture of a virulent strain of *Past. avisseptica* maintained here were subjected to the following tests and the results compared. The following statement shows the comparative results of the tests.

	<i>Past. aviseptica</i>	<i>Past. aviseptica</i>
(1) Cultural characters in broth	Izatnagar strain uniform turbidity	Madras strain uniform turbidity
(2) Motility (24 hours broth culture)	Non-motile	Non-motile
(3) Motility (under room temperature)	do.	do.
(4) Staining	Gram negative, cocco-bacillary	Gram negative cocco-bacillary
(5) Bile salt media	No growth	No growth
(6) Sugar reaction—		
Dextrose	+	+
Sucrose	+	+
Lactose	—	—
Mannite	+	—
Sorbito	+	—
Mannose	+	+
Salicin	—	—
Dulcitol	—	—
(7) Other biochemical reactions		
Indol	+	+
H ₂ S	+	—
V. P.	—	—
M. R.	—	—
Nitrate	+	+

(8) Biological

Twenty-four hours broth culture from the Madras strain inoculated into a rabbit killed it in 32 hours with typical lesions of *pasteurellosis* and numerous capsulated bipolar organism in blood smear, but the same culture produced only a slight febrile reaction when inoculated into a fowl and a duck. The experiment was repeated into fresh cultures isolated from the animal, but again there was no reaction. All attempts to increase its virulence by passing through rabbits failed.

(9) Cross immunity

It is possible that the organisms might have become avirulent due to cultivation in artificial media during transit from Madras to Izatnagar, and hence their

failure to produce any reaction in ducks. But such avirulent cultures are capable of conferring immunity as happens in *Past. bovis*. Therefore the fowl and duck originally inoculated with the Madras strain were tested for immunity after 11 days, with a 24-hour broth culture of the stock avirulent strain of *Past. aviseptica* (Izatnagar strain) and both the birds died within 24 and 36 hours respectively with typical lesions of *pasteurellosis*.

A rabbit was vaccinated with standard fowl cholera vaccine (from Izatnagar strain). After eight days the immunity was tested with a control rabbit using Madras strain. The control died within 36 hours and the vaccinated one within 48 hours. No immunity was produced though the action of the organism was a little delayed due to vaccination, both the strains being totally different from each other.

(10) An attempt was made to type the organism serologically. To avoid delay in submitting the report on typing, the laborious task of preparation of antiserum from rabbits was not carried out, but a shorter method was followed to give a fair idea of the type. A series of plate agglutination tests were carried out with the standard fowl cholera serum and antigens from (i) Madras strain (ii) Izatnagar strain and (iii) direct culture from rabbit which died after inoculation of Madras strain.

The following results were recorded :

(i) Rabbit culture readily agglutinated with the serum even when Izatnagar strain culture failed.

(ii) When thick emulsions of the organism in normal saline solution were added to fowl cholera serum, only rabbit culture was converted into clumps.

(iii) When emulsions of growth in fowl cholera serum itself were made, mixed and kept, all the three emulsions were broken up, but the rabbit one was more marked and quickly formed.

CONCLUSIONS

Serologically the organism seems to be a type of *Past. aviseptica*, a variant differing from the true type in its failure to attack sorbite and mannite and to produce hydrogen sulphide. It is pathogenic to rabbits only and not to fowls and ducks. Birds, recovered from inoculation, are not even immune to virulent strains of *Past. aviseptica*. This fact again gives room to doubt whether the strain isolated from ducks belongs to the type of *aviseptica* at all. It will not be out of place to mention in this connection that secondary invasions of *Pasteurella*, particularly in primary virus diseases, are not uncommon in birds, sheep and goats, and a serious epizootic of ducks, which broke out in Madras, and spread all over the military duck farms in India during the later part of the second World War, has already been recorded and there is every reason to conclude that this variant of *Pasteurella* is only a secondary invader.

SUMMARY

A culture of *Pasteurella aviseptica* isolated during an outbreak among ducks in Madras was received and subjected to cultural, biochemical and biological tests. It was found to be a variant of *Past. aviseptica* pathogenic only to rabbits and not to ducks and fowls.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. Vencheswaran, A.D.I.O. (Poultry), for the original cultures. Thanks are due to Mr. J. F. Shirlaw, Officer-in charge, Pathology and Bacteriology section, for his guidance and to Dr. S. Datta, Director, I.V.R.I., for the interest shown and encouragement given during the course of this investigation.

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THE EFFECTS OF HEAT TREATMENT AND LIME SEALING ON THE PRESERVATION OF SHELL EGGS

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(Received for publication on 1 June 1949)

ONE of the major causes of egg spoilage in India is the development of embryos in fertile eggs especially during the summer season. The eggs become inedible owing to the embryo growth after about 48 hours when high temperatures prevail. Although it is known that infertile eggs maintain their edible qualities much longer even under adverse conditions, the production of infertile eggs is a difficult problem under the present system of poultry keeping in India. Campaigns to produce infertile eggs have made little progress even in the U.S.A. except in areas where large flocks are maintained.

Funk [1943] developed a method of destroying the embryo in its early stages of growth by immersing the eggs in water or oil at constant temperatures for definite periods. The heat treatment of eggs was found to have the following beneficial effects on storage life: (a) many of the bacteria, if present on the shell and in the egg white are killed, (b) the embryo is killed and (c) the condition of the thick white and the yolk is stabilised. Funk observed that eggs heat-treated at temperatures ranging from 130°F. to 142°F. for a period of time just short of visual coagulation achieved the desired results. Bose and Stewart [1948] shed further light on the effects of heat-treating shell eggs and studied the possible complementary effect of oiling on heat-treatment (thermostabilization). They observed that heat-treatment in water at 130°F. produced a definite improvement in the initial albumen index of the 4-day-old eggs but not in the 5-hour-old eggs and indicated that the commonly accepted theory that egg quality of eggs which had undergone some deterioration, could not be improved by processing, was no longer tenable.

EXPERIMENTAL

Eggs. The day-old eggs for the experiment were obtained from the Institute Poultry Farm. They were laid by standard breed White Leghorn and Rhode Island Red pullets. The eggs were collected shortly after they were laid and held for a day in the cold store maintained at a temperature of 60°F. and relative humidity 90 per cent. Only eggs weighing 50-60 gm. were used for experiments and they were randomized in different groups to minimise differences in initial quality.

Processing of eggs. The eggs were placed in a wire basket and were heat-treated by immersing in a well-stirred water bath maintained at a temperature of 130°F. Bose and Stewart (*loc cit*) found that 130°F. is very convenient for heat-treatment of eggs because both the albumen and yolk could be heated to the bath temperature

without danger of coagulation. The lime-sealing was accomplished by soaking eggs in lime-water for about 18 hours. The lime water was made according to the method described in the *Poultry World* which is as follows: One pound of quick lime was placed in a container and one pint of boiling water was carefully added. After allowing the fluid to come down to the room temperature, $\frac{1}{2}$ pint of cold water and 4 oz. of powdered salt were added and stirred. The fluid was then strained and the clear fluid was poured over the eggs to be preserved. The eggs were kept in this fluid (lime water) for 18 hours, prior to preservation.

Storage condition. The storage consisted of placing the eggs at room temperature (May 1948, average temperature 92°F., average relative humidity 45 per cent) for 14 days and in the cold store (temperature 60°F. relative humidity 90 per cent) for 28 days.

Measurement of egg quality. The measurements of quality in eggs broken included (a) yolk index, (b) albumen index and (c) percentage of outer thin albumen.

Yolk index. One of the indications of decreased quality in eggs is a change in the size of the yolk. Water passes from the white to the yolk through the vitelline membrane and the size of the yolk is increased bringing about, thereby, a flattening of the yolk. The change in the relationship between the height and width of the yolk can be measured and has been used for judging egg quality. This ratio between the height and average width of the yolk is called the yolk index [Sharp and Powell, 1930]. In the present investigation the yolk index was measured with thick albumen intact, as it was found to be more convenient than the measurement of yolk index after removal of the albumen.

Albumen index. The thick albumen in the best quality eggs is concentrated around the yolk and tends to occupy the smallest possible area. As the quality decreases, the thick white becomes flatter and occupies more space. Heiman and Carver, [1936] devised the albumen index as a method of measuring egg quality. The index is determined by dividing the height of the thick albumen by the mean width.

Percentage of outer thin white. This was determined by drawing the thin albumen into a pipette and then measuring the volume of outer thin albumen thus recovered from each egg in a cylinder. The amount of apparent thick albumen (thick and inner thin albumen combined) was then determined in combination in the cylinder and the percentage of outer thin albumen calculated. The deterioration of quality in storage eggs should be judged by using a combination of the percentage loss of albumen index and increase in percentage of outer thin albumen, as an egg with a small firm albumen and a lot of watery albumen surrounding it does not present an appetizing picture.

As regards culinary properties it has been observed that the quality of cooked eggs varies directly with the physical measurement as detailed above.

RESULTS

The effects of the heat treatment and lime-sealing on the preservation of shell eggs are shown in the Table I. The eggs kept at room temperature during May 1948 for 14 days, without any treatment or processed in lime water only, were found

TABLE I
*Preservation of shell eggs (Day-old fertile eggs)**

Storage condition	Nature of treatment	Loss in weight per cent	Albumen index	Yolk index	Outer thin white per cent
Not stored	<i>nil</i>	—	0.095	0.46	38.5
Not stored	Heated in water at 130° F. for 15 to 60 minutes	0.51	0.110	0.46	31.9
Kept at room temperature during May 1948 for 14 days	<i>nil</i>	All eggs inedible due to embryonic development with blood formation			
	Lime-sealed	All eggs inedible due to embryonic development with blood formation			
Average temperature 92° F.	Heated in water at 130° F. for 15 to 60 minutes	9.23	0.073	0.26	35.2
Average relative humidity 45 per cent	Heated in water at 130° F. for 15 to 60 minutes and lime-sealed	2.89	0.083	0.31	37.4
Kept for 28 days in the cold store maintained at a temperature of 60° F. and relative humidity 90 per cent	<i>nil</i>	2.60	0.067	0.41	38.4
	Lime-sealed	1.17	0.075	0.42	42.1
	Heated in water at 130° F. for 15 to 60 minutes	3.22	0.098	0.40	36.6
	Heated in water at 130° F. for 15 to 60 minutes and lime-sealed	1.69	0.113	0.43	40.5

* Values for this series are averages for 12-24 eggs

inedible due to embryonic development with blood formation. Similar eggs kept for 28 days in the cold store maintained at a temperature of 60°F. and relative humidity 90 per cent were edible but the scores of the quality factors were rather low. Eggs, heat treated in water at 130°F. for 10 minutes or longer, killed the embryos and also stabilised the quality in shell eggs. The maximum benefit occurred when the eggs were heated for 15 minutes or longer. As there were no significant differences in the values of the quality factors for eggs heated in water at 130°F. for 15 to 60 minutes, the results were combined and the average values are recorded in the table. The heat treated eggs kept at room temperature (May) for 14 days were edible and only slightly inferior to the day-old eggs, whereas those kept for 28 days in the cold store at 60 F. virtually maintained the original quality of the eggs. The results on the combined effect of heat treating and lime-sealing are interesting in that they show a complementary effect of lime-sealing on the albumen and yolk quality of the heat treated eggs. The lime-sealing exerts its beneficial effect on keeping quality by preventing the escape of moisture and naturally-occurring carbon dioxide from the eggs. Sharp [1937] has emphasized the importance of the role of the naturally-occurring carbon dioxide in controlling the keeping quality of shell eggs. It has been shown that even small amounts of CO₂ effect yolk index retention favourably. The lime-sealed eggs also exerted a beneficial effect in reducing the percentage loss in weight of the storage eggs.

SUMMARY

Studies have been made to determine the comparative and complementary effects of heat treatment and lime-sealing on the keeping quality of shell eggs. The eggs without any treatment and also the eggs which were lime-sealed only, were found inedible owing to the development of the embryos with blood formation when stored at room temperature during the summer months. The heat treatment in water at 130°F. for 10 minutes or longer, killed the embryos and stabilized the thick white and yolk quality of the eggs. The maximum benefit occurred when the eggs were heat treated for 15 minutes or longer. The effect was quantitatively somewhat greater on albumen than on yolk quality. The lime-sealing exerted a complementary effect on the albumen and yolk quality of the heat treated eggs, by preventing the escape of moisture and naturally-occurring carbon dioxide through the shell pores.

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COMPONENT ACIDS OF COCONUT OIL

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IT is well-known that the character of the milk fat is markedly influenced by the character of its food source. Coconut cake is used to a large extent for feeding milch animals in places where coconuts are available in plenty. Hilditch and Sleightholme [1931], found that when liberal amounts of coconut cake were fed to cows, the butter fat showed a rise in lauric and myristic acids. Similar results were obtained by Anantakrishnan, Bhalerao and Paul [1947] by feeding 1.5 lb. of coconut oil as a supplement to milch cows and buffaloes. In this connection it was of interest to study the fatty acid distribution of coconut oil itself.

A number of reports on the component fatty acids of coconut oil have appeared from time to time, but none have shown the presence of lower unsaturated acids. Coconut oil is known to contain the lower saturated acids like caproic, caprylic, capric and lauric acids as shown by Armstrong *et al.* [1925], Collin and Hilditch [1928] and Longenecker [1939], but no mention is made of the existence of lower unsaturated acids like do-decenoic and tetra-decenoic acids. However, Longenecker [1939] has reported the presence of hexa-decenoic acid. The present paper deals with some observations on the component acids of coconut oil.

EXPERIMENTAL

Samples of coconut oil were collected from different parts of India and a composite mixture was analysed for its chemical constants. The results are shown below.

TABLE I

General analytical characteristics of coconut oil

Butyro refractometer value at 40°C.	35.6 Saponification value	257.5
Melting point	27.6 Reichert value	8.2
Iodine value	8.8 Polenske value	14.2

The sample was further subjected to a detailed analysis by ester fractionation according to the method of Hilditch as modified by Smith and Dastur [1938]. The methyl esters were obtained directly by refluxing with methyl alcohol and sulphuric

acid for 24 hours. The lower component acids were fractionally separated from the whole bulk, the higher members being separated into solid and liquid acids by Twitchell's lead salt method, methylated and also fractionally distilled. The results are shown in Tables II and III. In all 46 fractions were obtained. The fractions from 13 to 29 had low iodine values indicating the presence of unsaturated acids. As there was no probability of the oleic acid distilling at that low temperature of distillation and that it had been proved beyond doubt in case of butter-fat that the lower unsaturated acids distil over along with the saturated acids having the same number of carbon atoms, these fractions were suspected to contain the dodecenoic and tetra-decenoic acids.

TABLE II

Fractionation of the methyl esters prepared from 300 gm. of coconut oil

Fraction	B.P. at 2 mm. up to	Per cent of total esters	Molecular weight	Iodine values	Fraction	B.P. at 2 mm. up to	Per cent of total esters	Molecular weight	Iodine values
Lower esters									
1	Methyl butyrate	0.74	16	131	3.01	213.9	0.68
2	74	0.67	164.2	..	17	137	3.00	215.3	4.12
3	82	1.16	166.2	..	18	138	3.20	216.4	4.39
4	85	1.52	170.2	..	19	138	3.48	217.3	2.89
5	94	1.58	173.5	..	20	138	3.66	218.1	1.87
6	100	1.88	176.4	..	21	138	3.35	218.1	1.51
7	110	1.88	178.4	..	22	139	3.49	218.3	1.58
8	114	2.13	181.5	..	23	140	2.92	218.4	1.58
9	116	2.22	185.6	..	24	144	3.36	220.3	1.96
10	120	2.79	192.4	..	25	145	3.12	223.1	2.26
11	123	2.68	204.9	..	26	146	3.23	226.8	2.47
12	125	2.81	207.2	..	27	148	3.26	228.5	2.54
13	128	2.79	206.9	..	28	148	3.53	232.5	2.67
14	130	2.86	210.8	0.34	29	151	3.30	235.0	2.90
15	132	3.04	211.6	0.45	Total		76.06		
Solid esters									
30	130	1.25	232.4	0.15	35	158	1.68	262.0	1.81
31	145	1.32	241.2	0.24	36	158	0.77	271.4	4.05
32	148	1.64	243.5	0.41	37	165	2.10	281.7	4.54
33	152	1.43	250.7	0.62	38	Residue	1.61	303.5	13.67
34	155	1.66	253.4	1.09	Total		13.46		
Liquid esters									
39	127	0.60	226.6	4.63	44	162	1.88	291.7	87.94
40	153	1.00	241.7	20.69	45	162	1.70	291.7	91.83
41	157	0.89	249.7	24.22	46	Residue	1.58	295.2	91.92
42	159	1.12	260.2	50.41	Total		10.48		
43	160	1.71	288.2	81.93					

TABLE III

The component fatty acids and esters of the coconut oil

Acids	Per cent as methyl esters				Fatty acids (excluding unsaponifiables)	
	Lower	Solid	Liquid	Total	Per cent (wt.)	Per cent (molar)
Saturated						
C ₆	0.74	0.74	0.7	1.2
C ₈	4.28	4.28	4.2	5.9
C ₁₀	12.99	12.99	12.8	15.2
C ₁₂	44.19	0.42	0.31	44.92	44.8	45.8
C ₁₄	13.04	0.17	1.71	20.91	21.0	18.8
C ₁₆	..	4.19	0.88	5.07	5.1	4.2
C ₁₈	..	1.96	..	1.96	2.0	1.5
C ₂₀	..	0.23	..	0.23	0.2	0.2
<i>Total</i>	75.24	12.97	2.90	91.11	90.8	92.8
Un-saturated						
C ₁₂	0.35	0.35	0.4	0.4
C ₁₄	0.47	..	0.43	0.90	0.9	0.8
C ₁₆	0.78	0.78	0.8	0.7
Oleic	..	0.49	5.94	6.43	6.6	4.9
Linoleic	0.43	0.43	0.5	0.4
<i>Total</i>	0.82	0.49	7.58	8.89	9.2	7.2
Sum of the saturated and un-saturated acids	76.06	13.46	10.48	100.00	100.0	100.0

Identification of do-decenoic acid. Fractions 13 to 23 (C₁₂ concentrates) were mixed together and the fatty acids were recovered from them. They were subjected to lead-salt alcohol separation twice. A liquid acid fraction having a molecular weight 213.0 and iodine value 5.15 was obtained. As the quantity was not sufficient for the isolation of C₁₂ acid, it was oxidised with KMnO₄ in acetone. The fully saturated acid was freed from the acidic oxidation products by washing the ether solution with successive portions of 10 per cent K₂CO₃ and steam distilled. The equivalent weight was then calculated from the other extracts of the distillate

(212.4). It was found to be lower than that of the original acid indicating the presence of a unsaturated acid having lower molecular weight namely the do-decenoic acid. The results are shown below :

Fractions 13 to 23

	Original	1st lead salt	2nd lead salt
Saponification equivalent	217.8	213.1	213.0
Iodine value	2.00	3.18	5.20

Identification of tetra-decenoic acid. The fractions 24 to 29 were similarly mixed together and the fatty acids recovered from them. The acids were further subjected to repeated lead-salt alcohol separation till an acid having a molecular weight 227.6 and iodine value 108.2 was obtained, which very closely resembled tetra decenoic acid. The results are shown below :

Fractions 24 to 29

	Original	1st lead salt	2nd lead salt	3rd lead salt
Saponification equivalent	218.0	220.2	223.8	227.6
Iodine value	4.40	25.90	53.84	108.20

DISCUSSION

It can be seen from Table I that coconut oil has high Saponification and Polenske values indicating the presence of more of glycerides of caproic, caprylic and capric acids, which is confirmed by the results shown in Table III. The chief constituents of coconut oil are capric, lauric and myristic acids of which lauric forms about 45 per cent and confirms the findings of the previous workers. Do-decenoic and tetra-decenoic acids are also found to exist in small proportions.

SUMMARY

Coconut oil has been analyzed for its chemical characteristics and the fatty acid composition by fractionation through an E.H.P. column.

It is found to contain capric (0.7, 1.2), caprylic acid (4.2, 5.9), capric (12.8, 15.2), lauric (44.8, 45.8), myristic (21.0, 18.8), palmitic (5.1, 4.2), stearic (2.0, 1.5), arachidic (0.2, 0.2), do-decenoic (0.4, 0.4), tetra-decenoic (0.9, 0.8), hexa-decenoic (0.8, 0.7), oleic (6.6, 4.9), and linoleic (0.5, 0.4) acids by weight and molar percentages.

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ABSTRACTS

Studies on the Role of Cobalt in Sheep Nutrition. RAY, S. N., WEIR, W. C., POPE, A. L., BOTHSTEDT, G., AND PHOLES, P. H. (1948). *J. Ani. Sci.* 7 (i) 3-15

FIVE groups of cobalt deficient young lambs were used for the experiment. The control group received only the cobalt deficient basal ration while the other groups received besides the basal ration supplements of 4.55 mg. thiamine, 2.6 mg. folic acid, 100 mg. of pyridoxine (all by injection) and 17.5 mg. of cobalt sulphate (by oral administration) twice weekly. The experiment lasted for a period of nine weeks and the effect of the vitamin and cobalt supplements on the average weight and haemoglobin content was studied.

After about five weeks the control animals as well as those receiving folic acid and pyridoxine supplements began losing weight, whereas, thiamine and cobalt groups consistently gained weight. Cobalt and thiamine improved the appetite of the animals and a gain in weight followed. Animals which received cobalt supplements showed the maximum increase in body weight (29.0 lb. average) followed by those of the thiamine group (16.8 lb. average). The pyridoxine group which consumed the same amount of concentrate did not produce a proportionate increase in body weight. By comparison injection of cobalt salts produced a much slower response.

It was noticed that all the groups suffered a decrease in haemoglobin values. The cobalt group, both injection and oral, showed the least decrease but even here the final figures were well below the average. Severe anaemia developed in thiamine treated animals which showed some improvement when thiamine was stopped.

The livers of the cobalt deficient lambs were hypertrophied and high in fat content. Administration of 1 mg. cobalt per day for seven weeks improved the condition. The cobalt content of the livers of these animals was very low but could be brought back to the normal level by seven weeks cobalt administration of 1 mg. per day.

The effect of cobalt on appetite seems to be direct, and that upon haemopoietic centres indirect. It is presumed that the beneficial effect of cobalt is linked up with the normal production of the members of the vitamin B complex in the rumen. (T. M. P.)

The Significance of Certain Bacteria in Pasteurised Milk. SPECK, M. L. (1948). *Milk Plant Monthly* 37, 36

THE practical significance of the occurrence of some of the bacteria, particularly of micrococci, thermophilic, coliform and of the microbacteria in pasteurised milk has been discussed in this article by the author.

The micrococci derived either from the udder or from soil may be small in number, although in unclean utensils or processing equipments (where temperatures are favourable for their growth, particularly soon after washing and the long interval before use) they may cause in pasteurised milk, serious trouble, in its compliance of the bacteriological standards. The presence of these organisms in large numbers in pasteurised milk is suggestive of unclean methods practised during washing of equipment with which the milk comes into contact during processing.

The author points out the thermophilic bacteria entering raw milk find full scope for their growth during pasteurisation of the first batch of milk in holding vats, since the temperature used in this type of pasteurisation is conducive for their growth. The remaining milk in the vats acts, as an inoculum for the succeeding batches of milk. Unless, therefore, the whole plant is dismantled and cleaned carefully with hot chlorine water at 180-190°F. the milk pasteurised in these vats will develop high acidity. Since H.T.S.T. pasteurisation temperature does not allow these organisms to grow and the milk is not held at the elevated temperature for long, the thermophilic contamination is rarely met with in this type of pasteurisation.

The coliform bacteria which are mainly derived from the intestines of the animals get into milk and their number in raw milk is inversely proportional to the care used in milking operations. These are killed by proper pasteurisation. Hence their presence in pasteurised milk affords a clear indication of (1) improper pasteurisation or, (2) post pasteurisation contamination due to faulty handling of milk. Improperly sterilised pipe joints, valves, cooler, filter, etc. are some of the sources of these organisms.

The microbacteria which are highly heat resistant gain entry into milk from soil, manure and improperly cleaned utensils. These have the same significance as the micrococci.

The occurrence of any of these organisms in large numbers is to be taken as a warning that more serious trouble may result if steps are not taken for correcting faulty practices which permit the presence of these organisms.

Four photomicrographs of the types of micro-organisms discussed above are also given. (K.V.)

A Comparative Study of the Biochemical Activity of *Streptococcus Lactis*, *Streptococcus Citrovorus* and *Streptococcus Paracitrovorus* when Grown in Cows Milk and Soybean Milk. GEHRKE, C. W., AND WEISER, H. H. (1948). *Jour. Dairy Sci.* 31 (4) 213-222

THE authors reviewing the work of various investigators on organisms used in butter cultures point out that *S. lactis* primarily attacks lactose, forming large amounts of lactic acid while the other associated organisms bring about the decomposition of citric acid to form volatile acids and compounds like diacetyl and its precursor acetyl-methyl-carbinol, these together contributing to the characteristic aroma of butter.

A comparative study of the biochemical activities, titratable acidity, pH, volatile acidity, diacetyl and acetyl-methyl carbinol, has been reported on samples of cow's milk and soybean milk inoculated with butter culture organisms namely *S. lactis*, *S. citrovorus* and *paracitrovorus*.

The values obtained for pH and volatile acidity were comparable in most instances for both types of milk held at various incubation periods, extending up to 216 hours. But cultures inoculated in soybean milk and incubated at 21°C. had an average lactic acid content of only 0.51 per cent. Whereas cultures inoculated in cows milk had an average lactic acid content of 1.05 per cent when incubated under similar conditions. The values obtained for the titratable acidity in cow's milk and cow's milk to which 0.15 per cent citric acid was added were nearly twice as great as those secured for the cultured soybean milk even when citrate was added to it. Early during ripening pronounced changes in titratable acid or pH had little effect on the amount of acetyl/methyl carbinol plus diacetyl present, but later, significant increases occurred with little or no change in acidity.

Acetyl-methyl-carbinol and diacetyl were developed only slowly in soybean milk and the results were similar to those obtained with cows milk only after prolonged incubation. The total acetyl-methyl-carbinol and diacetyl after 24 hours of incubation at 21°C. was nearly three times as much in the case of cow's milk as that in soybean milk. (S.N.A.)

Dye Reduction Tests in the Bacteriological Examination of Dried Milk. HIGGINBOTTAM c. (1948). *Jour. Dairy Res.* 15 (3) 280-284

THE possibility of applying dye reduction tests, for assessing the keeping quality of reconstituted milk after ageing, to get a convenient reduction time has been investigated.

Over 100 samples of roller dried full-cream milk and about 100 samples spray dried milk were reconstituted and incubated for 20 hours at 55°, 37°, 30°, 22° and 15.5°C. respectively. They were then examined with methylene blue and resazurin reduction tests and the results compared with the plate counts of freshly reconstituted milk on yeastral milk agar at 30 and 37°C. and also with the keeping quality at 15.5°C. Ageing at 22°C. was found most convenient giving a mean methylene blue reduction time of 2½ to 3 hours for spray dried milk and 3 to 3½ hours for roller dried milk. They however obtained a very poor correlation with plate count at either 30 or 37°C. and with keeping quality test made at 15.5°C. Ageing at 55°, 37° or 30°C. correspondingly gave shorter reduction intervals, delayed and points and clotting at the time of examination. Incubation at 15.5°C. gave methylene blue reduction time of over six hours for about 50 per cent of the samples and even those which reduced within six hours failed to give a fair correlation with plate count or keeping quality tests. The results with resazurin reduction were similar to those obtained for methylene blue. The temperature of reconstitution, viz., at room temperature or at 50°C. showed no difference.

The author, therefore, concludes, that though ageing at 22°C. gave a convenient range of reaction time, due to lack of correlation with plate count and keeping quality, the results cannot be accepted as giving a true assessment of the bacterial quality of the milk, or more strictly, an assessment bearing any relation to the present accepted plate count technique. (V.K.N.N.).

Effect of Season, Breed and Species of Ruminants on the Vitamin A Potency of Butterfat. SARKAR, B. C. RAY (1948). *Jour. Dairy Sci.* 31, 165

THE seasonal variation of the carotene and vitamin A potency of butterfat of Haryana cows and its relationship to the intake of carotene in green fodder has been studied. The maximum total vitamin A potency of 24,734 I.U. per lb. was reached during July, August and September comprising the Monsoon season and the minimum value of 16,093 I.U. of vitamin A was reached during November, December and January. These fluctuations in carotene and vitamin A of butterfat could be correlated to the level of carotene ingestion and it is calculated that a daily intake of 45 lb. of average green fodder per cow could maintain the maximum vitamin A potency throughout the year. The stage of lactation has been found to have very little effect on the vitamin A potency of butterfat.

It has been found that neither Reichert-Meissl number nor Saponification value of the butterfat is influenced by the feeding of green fodder while there is an increase in Iodine value when green fodder is included in the ration. There has been very little change in the general composition of milk throughout the experimental period except for 25 per cent increase in fat during November, December and January. The butterfat of goats and buffaloes contained only traces of carotene while the total vitamin A potency of goat milk was equal to that of cow milk and that of buffalo milk was comparatively low. (A.K.).

Effect of Water Sprinkling With and Without Air Movement on Cooling Dairy Cows. SHEATH, D. M. AND MILLER, G. D. (1948). *Jour. Dairy Sci.* 31, 361.

THE trials showed that the least cooling of cows took place without any sprinkling or air circulation and the best results were secured when cows were first sprinkled and then subjected to air circulation. The use of sprinkling alone or fan alone produced essentially equal results, while sprinkling produced the greater drop in both body temperature and respiration rate at the end of 0.5 hour and the fan, being the more effective by the end of one hour. In these comparisons it is of interest that sprinkling alone produced a lower respiration rate at the end of 0.5 hour than was present after one hour. This was not true for body temperature, which was much slower to respond to cooling treatment. The fact, that the cows were sprinkled just once and with water at approximately 85°F., probably helps to explain why the maximum reduction in respiration was secured at the end of the 0.5 hour period.

Shade alone showed a small change in that direction, while the fan alone and sprinkling alone were intermediate in their effects.

The results of this experiment give valuable information on how rain and wind as produced by nature tend to cool milking cows during summer months. The results also suggest the need for further experimental work on how mechanical sprayers and fans may be utilized economically during summer when nature is not producing wind or rain. (D.N.)

Egg Records as a Criterion for Selecting Breeding Hens. HAYS, F. A. (1946).
Poult. Sci. 25, 622-27

THE first part of the article consists of an exhaustive at the same time interesting review of the pioneer work on breeding for egg production. In general the balance of evidence indicated that annual egg production depended upon a complex of internal and environmental factors, as such gross egg production of dams is a poor criterion of their transmitting ability.

A study made by the author on Rhode Island Reds at the Massachusetts Agricultural Experiment Station over a period of seven years; classification of dams and daughters having been made with respect to inherited characters, such as, early maturity, intensity of laying, absence of winter pause, non-broodiness, persistency of production at the end of the first year, revealed certain interesting features. These are:

In the first year production the correlation between mothers and daughters, was of little biological significance. There was, however, slight but significant increase in daughters' production as the production records of the dams increased. The higher and lower producing hens differed mainly in good or poor egg production during the winter months, in other words, presence or absence of winter pause; similar differences were again observed in daughters from high producing hens when compared with daughters of low producing hens.

He concluded that good winter egg production is a valuable and promising criterion of the breeding worth of hens.

(In Northern India hens used as breeders should lay well from November to March.) (S.G.I.)

Streptomycin, in Experimental Infections and Its Possible Use in Veterinary Therapeutics. KARLSON, A. G. AND FELDMAN W. H. (1947). *J. Amer. vet. med. Ass.* 110, 63-70

STREPTOMYCIN, the latest wonder drug, owes its origin to the organized efforts of Wakesman and collaborators in their excellent attempts to discover an antibiotic substance with a wide therapeutic ratio. It is a white powder, markedly stable and highly soluble in isotonic saline solutions. The optimal pH for its full action is 9.0 and when the pH is lower than the neutral point, it becomes less active.

The unit of streptomycin is an S unit. Mice can tolerate as much as 10,000 S units in a single subcutaneous injection while 100 S units are sufficient to protect them against a lethal inoculum *Salmonella schottmulleri*.

In vitro studies indicate that streptomycin is bacteriostatic for a wide variety of bacteria that cause disease in animals. It should be remembered, however, that the activity of a drug *in vitro* may be no measure of its activity *in vivo*.

In vivo, streptomycin is effective in controlling experimental infections by various Gram-negative bacteria and tubercle bacilli. Tularemia in men, brucellosis in men and guinea pigs and pneumonia in men and mice have been successfully treated with streptomycin. Experimental leptospiral jaundice in dogs is controlled by this drug. The drug is not active against fungi, trypanosomes and malarial parasites nor can it inactivate tetanus toxin. It has no effect on the few viruses tried.

Streptomycin exerts a marked activity in resolving or suppressing established tuberculosis in the guinea pig. In the treatment of human tuberculosis this drug has so far, given very encouraging results.

In veterinary practice large animals may not be sufficiently valuable to be treated for prolonged periods with this drug. Preliminary toxicity studies are essential in animals due to the differences in the metabolic processes and these should be taken up by the veterinarians. A high concentration of this drug in the blood stream will have to be built up and this requires its injection every three or four hours in sufficient bacteriostatic concentration over long periods. If frequent injections become necessary to treat large animals, the usefulness of streptomycin in veterinary practice will obviously be limited. The drug is now in great demand for the treatment of human tuberculosis and it is unlikely that it will become available for veterinary practice for a long time to come. Moreover, for large farm animals, the use of this drug is not economically feasible. The drug, can, however, be used in pet animals like dogs and cats.

The necessity for frequent use of the drug can be overcome by the use of a vehicle to delay the absorption of the drug and investigation is required to discover such a vehicle.

One should not advocate the use of this drug in the treatment of bovine tuberculosis, anthrax, glanders in horses and rabies in animals. In the case of valuable animals an attempt may be made to treat bovine brucellosis though chronic forms of this disease do not respond to this therapy. As the drug is excreted in high concentration in the urine in an active state it may be used in the treatment of urinary infections of herbivorous animals which have an alkaline urine, e.g., in pyelonephritis of cattle.

In bovine actinomycosis and actinobacillosis this treatment may be combined with surgical intervention. When administered orally the drug is not absorbed in the blood but remains in an active state in the intestines and when given in sufficient quantity is capable of sterilizing the faeces. This fact can be taken advantage of in the treatment of bacterial enteritis in animals, especially as the drug is non-toxic, antibacterial in nature and by reason of its stability can be placed in feed

and water. If cost is no consideration, it can be extensively used in poultry practice as a prophylactic measure against diarrhoeal diseases.

In bovine mastitis, streptomycin may prove to be an adjunct to penicillin in the treatment of mixed infections. Work should be carried out with a view to collect data concerning the excretion of streptomycin in the milk following parenteral injection in animals; as it is important to determine whether effective milk levels may be maintained in cattle by intramuscular or subcutaneous injection of the drug. The alkaline reaction of mastitis milk should prove an ideal and suitable medium for the activity of this antibiotic. If and when streptomycin becomes available for veterinary practice efforts should be made to base the field trials on actual laboratory evidence together with studies regarding the dosage, mode of administration, toxicity rate of elimination and the effect on the diseases treated. (P. R. K. I.)

Symposium on Exoerythrocytic Forms of Malarial Parasites. III. The Chemotherapy of Malaria in Relation to Our knowledge of Exoerythrocytic Forms. COATNEY, R. G. AND COOPER, W. C. (1948). *J. Parasit.* **34**, 275-289

THE authors have studied the effect of various chemotherapeutic agents on avian malaras where exoerythrocytic forms have actually been demonstrated and in simian and human malaras where presence of E-E has been deduced from indirect evidence. At the time of writing this paper these authors were not aware of the findings of Shortt and his collaborators relating to E-E forms in simian malaria.

Plasmodium cynomolgi and human malaria, *P. vivax*.

In avian malaras (*P. gallinaceum* in the chick, *P. cathomerium* and *P. relictum* in the canary and *P. lophurae*, in the chick, canary, duck and turkey) certain sulfonamides, naphthoquinones, acridones, s-triazines and one other compound, a pyrimidine were found to produce protective effects which could be attributed to action against pre-erythrocytic stages of these parasites. One naphthoquinone derivative (SN 8557), however, was found to cure an established infection where E-E forms were known to occur.

As for simian malaria due to *P. cynomolgi* infection it is shown that representative 8-aminoquinolines with or without concomitant quinine had curative effect on the sporozoite induced *cynomolgi* infections.

In the case of human malaras two drugs, 8-aminoquinolines and biguanides are shown to have activity against the hypothetical E-E forms. Both prophylactic and curative action of 8-aminoquinolines against *P. falciparum* and *P. vivax* is demonstrated; while paludrine (a biguanide) though possessed prophylactic action against *P. falciparum* and *P. vivax* was not able to cure an established *vivax* infection. (H. N. R.)



REVIEW

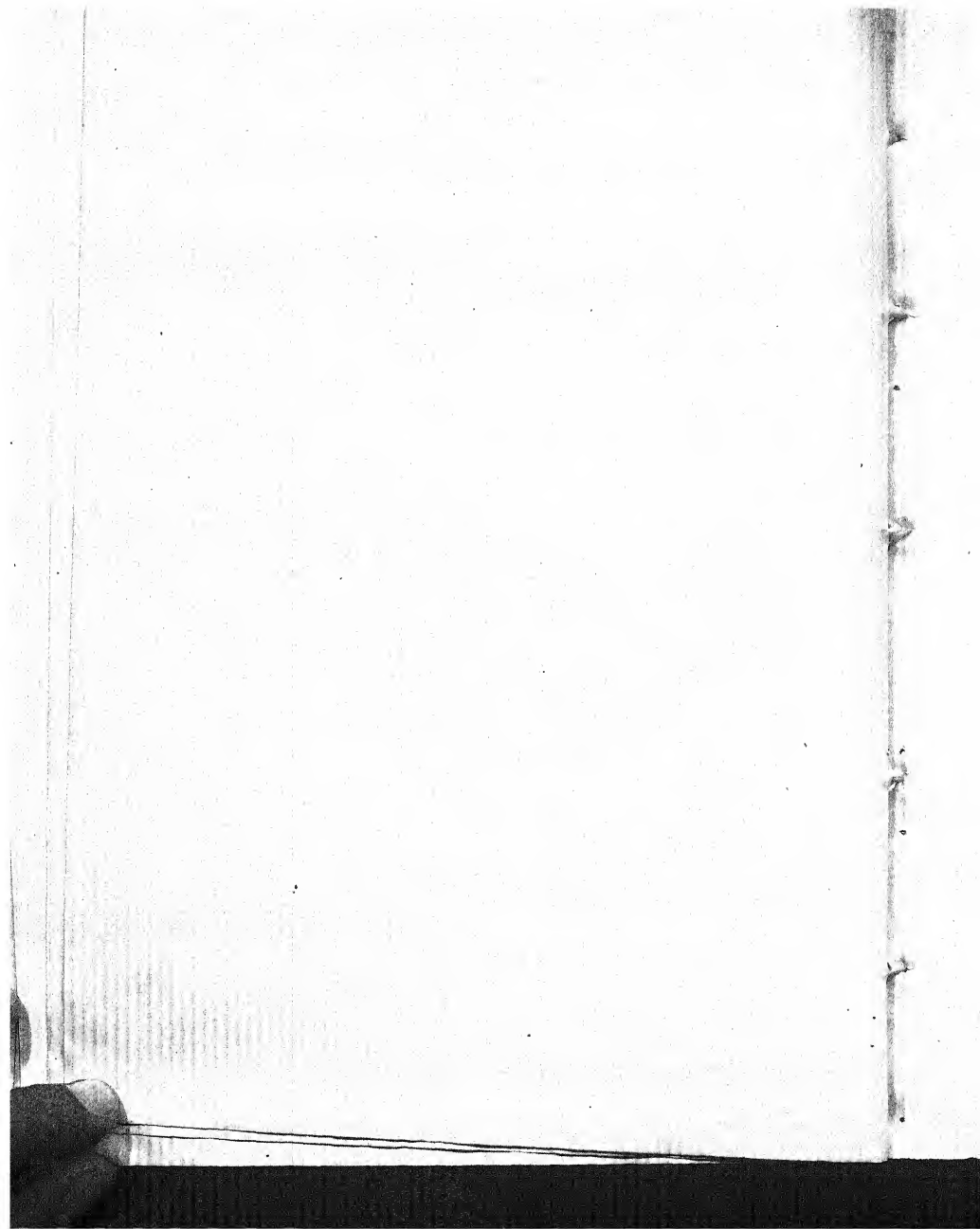
Dictionary of Genetics

By R. L. KNIGHT. (Published by the Chronica Botanica Co., Waltham, Mass., U. S. A., and Macmillan and Co., Ltd., Calcutta, 1948, pp. ix and 183, \$4.50)

THE Dictionary of Genetics, prepared by Dr Knight, the Senior Economic Geneticist to the Empire Cotton Growing Corporation and to the Sudan Government, is the second volume of the '*Lotsaya*—Biological Miscellany' series being published under the editorship of Dr Frans Verdoorn. In the words of the author, this dictionary is an attempt to define and standardize technical terms used by workers in the field of Genetics. This volume contains an extensive glossary of terms used in Genetics, and allied subjects like Cytology, Animal Breeding, Heredity and Evolution. The glossary has not been limited solely to these terms. The scope of the book has been enlarged by including terms used in Reproductive Physiology and Embryology. Chemical terms used in some of the above subjects have also been included. In all, there are about 2,500 terms which have been defined. On page 63 has been given information about the gestation period of different mammals and on page 123 the various types of reproductive mechanism have been detailed in a schematic form. The appendices contain important formulae of Biometry required in Genetics and Plant and Animal Breeding with six relevant tables. The appendices also contain the international rules for symbolising genes and chromosome aberrations, and a table giving information regarding the distance recommended to avoid seed contamination. At the end is given a bibliography of books and scientific papers made use of in compiling the dictionary.

This book will surely remove a gap in the field of Genetics. The need for such a publication was keenly felt as the glossaries hitherto compiled by other geneticists appearing as appendices to texts or in scientific periodicals were rather brief and inadequate to meet the all-purpose need of this young but very rapidly developed science. As has been pointed out in the preface of the book the publication of such a comprehensive dictionary will help in understanding the Genetic literature more readily by the usage of existing terms instead of coining unnecessary new words. The inclusion of both modern and older terms will help the coiners of new words to avoid putting an entirely new meaning to old established terms. These will remove the present confusion in the Genetical vocabulary.

This dictionary will invariably establish itself as an invaluable aid as a reference book to the students and workers in Genetics and allied Science; the author has done a great service to geneticists in general. A copy of this publication will be a valuable reference book for every worker in Genetics and allied sciences. (P. B.)



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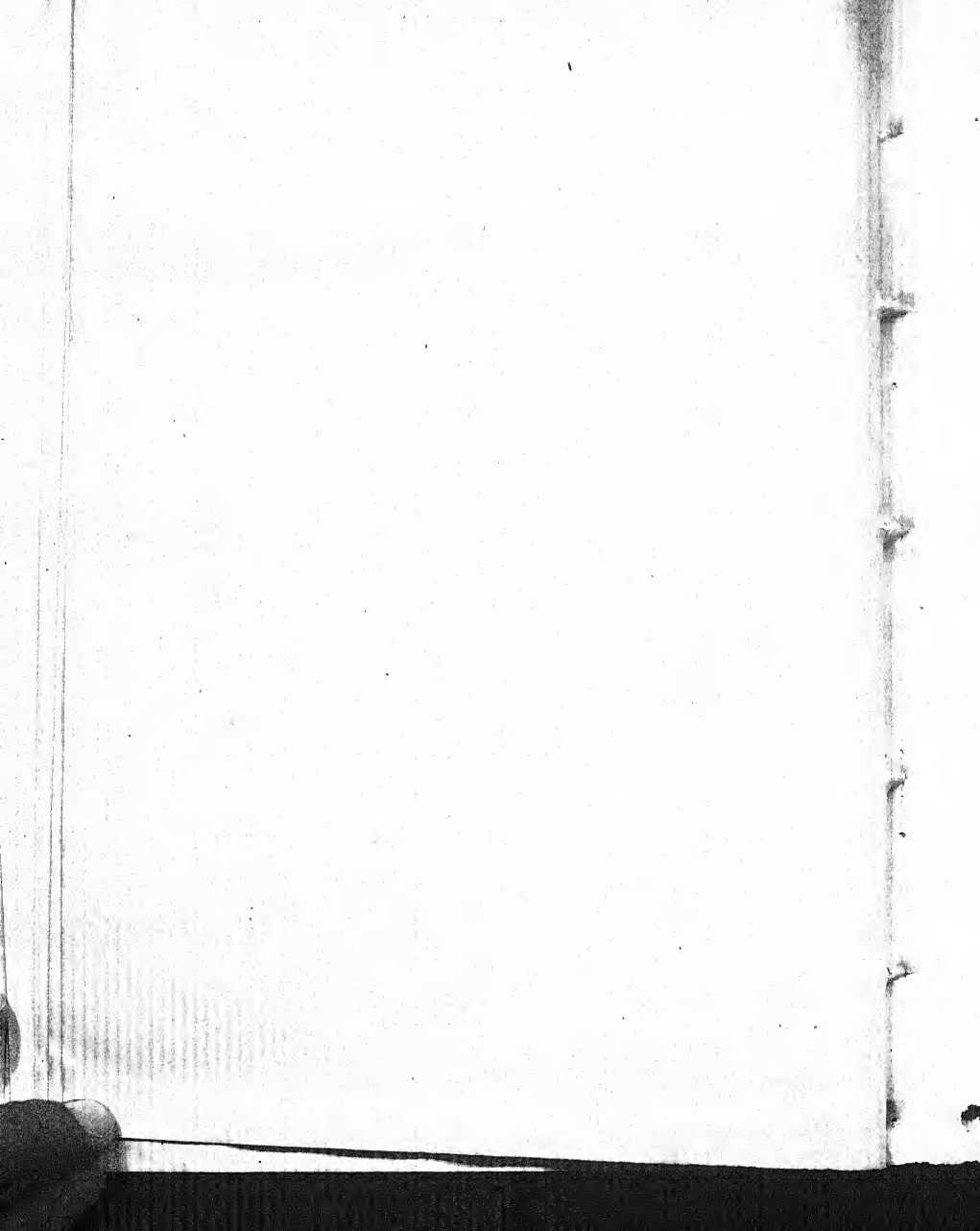
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ORIGINAL ARTICLES.

NATURE AND DIAGNOSIS OF *PASTEURELLA* INVASION IN BLOOD CIRCULATION

By R. N. NAIK, G. B. V. C., Bacteriologist and Vice-Principal, I/C Patho-bacteriological Laboratory, Bombay Veterinary College, Bombay.

(Received for publication on 11 March 1949)

(With four text-figures)

PASTEURELLOSIS is one of the most important diseases of great economic significance prevalent in domestic animals in India. It stands next to rinderpest in causing high mortality in animals especially buffaloes and cattle. It is prevalent in certain regions seasonally. It breaks out first in buffaloes or at least in a larger number of buffaloes than in cattle. The number of animals attacked in each outbreak is smaller than that in rinderpest; but what matters most for stock-owners is that their animals, young in age and robust in physique are attacked all of a sudden and succumb, allowing little time to fetch proper aid. Diagnosis of the disease is not always easy under field conditions due to the occurrence of the disease in various forms, in some of which characteristic symptoms are not present, the absence of dead cases available for post-mortem examination at the time of the Veterinary Officer's visit and the frequent prevalence also of other infectious diseases such as anthrax, bovine surra, black quarter and dengue in the same region. Veterinary Officers, therefore, forward to the Provincial Diagnostic Laboratory blood smears from affected or dead cases suspecting very often two or more diseases. In pasteurellosis the microscopic examination of blood smears collected at any stage of the disease does not always reveal the causal agent as in the early stages of anthrax. Besides, the smears collected from putrefied carcasses are not dependable for declaring a definite diagnosis. With a view, therefore, to improving the method of laboratory diagnosis of this disease a comparative preliminary study was made in the course of which was found that in many cases in which specimens of blood were positive biologically, blood smears were negative microscopically. This fact necessitated a study by means of microscopic, cultural and biological examinations regarding the invasive power of *Past. bovisseptica* in the blood circulation from the time of its introduction until death of the host. This article incorporates the results of this study.

HISTORICAL

It has been stated in literature on bacteriology that pasteurellosis could be easily diagnosed microscopically. Kelser and Schoening [1943] consider that in all positive cases, bipolar organisms are found in teeming numbers. Gaiger and Davis [1947] state that in the bodies of animals which have died of acute infections the organisms are easily found microscopically in films made from blood, the excretions and the parenchyma of the internal organs. Hutyra and Marek [1946], however, say that occasionally bacteriological examination is rendered difficult by the absence

of the bacilli from the blood in pasteurellosis of cattle, and the presence of only a few bacilli in the exudate. In the case of the disease in buffaloes, they state, however, that *Pasteurella* is easily found in the blood. Bennet [1926], with the experience of the Indian Institute of Veterinary Research, Mukteswar, gives a real picture when he says, 'It should be remembered, however, that in some cases of haemorrhagic septicaemia, the bacteria are found in the blood only with extreme difficulty and after a prolonged examination of the blood smears and in such cases the rare occurrence of the bacteria (if the carcass is fresh) is almost differentially diagnostic of haemorrhagic septicaemia, since in anthrax the bacilli nearly always occur in large numbers'. This observation indicates that even in cases which have died of *Pasteurella* the bacilli are sometimes absent and that laboratory workers too might have possibly confused this disease with anthrax in the past.

In these days, at the time of outbreaks, the field staff find a large number of suffering cases than dead ones. The disease cannot be diagnosed definitely from clinical symptoms alone due to reasons stated already. Dead cases are often putrefied due to the prevalent tropical conditions. It is, therefore, sometimes necessary to diagnose the disease from the material collected from suffering cases. Very few workers in the past have attempted any exhaustive work on the occurrence of *Pasteurella* in the blood of living cases during the different stages of the disease.

EXPERIMENTAL

Strains of *Past. bovisepctica* which were recently isolated from haemorrhagic septicaemia cases in this laboratory and which were sent to the Indian Institute of Veterinary Research, Mukteswar, were used for conducting the experiments.

Experiment I. Nature of Pasteurella invasion in the blood circulation as determined by the microscope

Nine rabbits were subcutaneously infected with *Past. bovisepctica* as detailed in Table I. Blood smears which were collected from the tip of the ear of the rabbits every hour from the time of infection until their death, were stained by Geimsa's stain for 30 minutes and examined microscopically for the presence of *Pasteurella* organisms with the results indicated in Table I.

Determination of facts

1. Out of nine rabbits infected with haemorrhagic septicaemia five died within 24 hours and the rest in 48 hours.
2. *Pasteurella* organisms were detected microscopically one hour before death in all nine cases; two hours before death in five cases; three hours before death in four cases; and six hours before death in one case.
3. No *Pasteurella* was detected microscopically prior to six hours before death in all nine rabbits.

Experiment II. Biological tests into blood samples of infected rabbits during life

The findings of Experiment I were rather surprising. It was difficult to believe that in spite of the septicaemic affection produced by the *Pasteurella* as

TABLE I

Pasteurella invasion in the blood circulation as determined microscopically

Rabbit number	Source of <i>Pasteurella</i>	Date and time of infection	Date and time of death	Interval between infection and death	Time of detection of <i>Pasteurella</i> in blood smears	Number of <i>Pasteurella</i>	
						during life	after death in heart blood
218	Blood of bull, 3 years Baramati, District Poona	17-8-1948 at 2 p.m.	17-8-48 at night	Not determined as it died at night		Many	
203	Blood of buffalo cow calf, 1 year Hirekurr, District Dharwar	21-8-1948 at 8-10 a.m.	21-8-1948 at 8-55 p.m.	12-45 hours	2-45 hours before death	Rare at 6-15 p.m. and many until death	Many
209	Blood of bull, 2 years of Vani, District Nasik	21-8-1948 at 8-15 a.m.	22-8-1948 at night	Not determined as it died at night		Many	
213	Blood of aged buffalo cow of Rahimatpur, District Satara	24-8-1948 at 10-30 p.m.	25-8-1948 at 7-45 p.m.	21-15 hours	5 hours before death	Rare at 4 and 5 hours before death and numerous later on	Many
240	Blood of buffalo bull calf, 2½ years of Vani, District Nasik	1-9-1948 at 4 p.m.	2-9-1948 at 5 p.m.	25 hours	1 hour before death	Rare	Few
219	Blood of bullock of Hirekurr, District Dharwar	1-9-1948 at 5 p.m.	3-9-1948 at 1-15 p.m.	44-15 hours	2 hours before death	Very Rare	Very few
221	<i>Pasteurella</i> culture isolated from rabbit No. 240 <i>supra</i>	14-9-1948 at 4-45 p.m.	15-9-1948 at 11 a.m.	18-15 hours	2 hours before death	Rare	Rare
225	<i>Pasteurella</i> culture isolated from rabbit No. 218 <i>supra</i>	14-9-1948 at 4-45 p.m.	15-9-1948 at 5-45 a.m.	13 hours	1 hour before death	Rare	Rare
238	<i>Pasteurella</i> culture isolated from rabbit No. 203 <i>supra</i>	14-9-1948 at 4-45 p.m.	15-9-1948 at 10-16 a.m.	17-30 hours	2 hours before death	Rare	Rare

NOTES.—1. Smears prepared after death from the spleen, lymphatic gland and the sent of inoculation showed *Pasteurella* in large numbers but those prepared from other vital organs and the peritoneal exudate a fair number.
 2. Cultures made from the heart blood after death gave rise to a profuse growth of *Pasteurella* in culture media in all cases.

indicated by the rise of body temperature, dullness and loss of appetite, *Pasteurella* could not be detected in the blood circulation prior to six hours before death. It was believed that organisms were present but due to their small number and their presence in some indistinguishable forms like faintly stained cocci, they could not be detected microscopically. With a view to confirming this idea two rabbits, Numbers 228 and 238, were injected subcutaneously with virulent *Pasteurella* on 28-9-1948 at 12 p.m. Blood samples and blood smears were collected from them in the morning every hour from 6½ hour onwards after infection. Both of them died between 9 and 9-30 p.m. on the same day, i.e. about 22 hours after infection. Blood smears collected from them proved positive for pasteurellosis from 3-30 p.m. onwards, i.e. six hours before death. Blood samples collected at 6-30 a.m. and 11-30 a.m. were tested for pathogenicity by injecting into rabbits with a positive result in each case.

This experiment revealed that the blood samples collected at the tenth and fifteenth hour before death, proved positive for *Pasteurella* infection in the biological test, while blood smears collected at the same time failed to reveal any distinguishable forms of *Pasteurella* in the microscopic examination.

Experiment III. Pasteurella invasion in the blood of rabbits during life as determined by the cultural and animal inoculation tests

As the findings of the preceding experiment have not been reported previously, the question was further pursued in order to find out how soon, after infection, the blood of rabbit infected with *Pasteurella* becomes invaded by the organism. Two rabbits were subjected to the test; the results are indicated in Table II.

TABLE II

Cultural and pathogenicity tests into blood of artificially infected rabbits during life

Time of bleeding of infected rabbits	Rabbit No. 204 infected with <i>Pasteurella</i> strain, R. 218		Rabbit No. 205 infected with <i>Pasteurella</i> strain, R. 209.	
	Culture with a loopful of blood	Rabbit inoculation with 0.5 c.c. blood	Culture with a loopful of blood	Rabbit inoculation with 0.5 c.c. blood
	Infected with <i>Pasteurella</i> at 9-0 a.m. on 7-10-48			
10-15 a.m.	No growth	Not infective	No growth	Not infective
11-15 "	11 colonies of <i>Pasteurella</i>	Infective	do.	Infective
12-15 p.m.	35 do.	do.	do.	do.
1-15 "	102 do.	Not done	10 colonies of <i>Pasteurella</i>	Not done
2-15 "	140 do.	do.	18 do.	do.
3-15 "	150 do.	do.	Contaminated	do.
4-15 "	206 do.	do.	38 colonies of <i>Pasteurella</i>	
5-15 "	202 do.	do.	91 do.	do.

Further observation was not made. Both died at night the same day. Heart blood cultures made next morning were positive in each case.

Determination of facts

1. *Pasteurella* invasion occurred in artificially infected rabbits 2·15 hours after infection.
2. The above invasion was possible to be determined only by the animal inoculation test in both cases and by the cultural test in one case.
3. The colony count of the blood showed a progressive increase in the *Pasteurella* colonies every hour as the septicaemia progressed.

Experiment IV. Nature of Pasteurella invasion in the blood circulation of cattle and buffaloes

The natural facts determined in the preceding three experiments were of immense importance in the diagnosis of haemorrhagic septicaemia in the field where immediate control measures were required to be applied. Since we were called upon to deal with outbreaks of haemorrhagic septicaemia in large animals whose natural resistance was greater than that of rabbits, it was deemed necessary to repeat these experiments in buffaloes and cattle. Accordingly, three buffalo calves and one bull calf were infected with mixed *Pasteurella* culture of strains R. 216 and R. 226 (*supra*) at 10·15 a.m. on 25·11·1948. The dose used was 0·5 c.c. of blood agar culture tube emulsified in 4 c.c. of normal saline. The body temperature of each of these animals was noted and also blood and blood smears were collected from them every three hours day and night from the time of infection until death. The blood was collected with aseptic precautions from the jugular vein and a drop of it was transferred directly from the syringe into a culture medium and the remaining blood stored in ice for the purpose of conducting animal inoculation tests. The details of the various tests carried out are given in Table III.

Determination of facts

1. The incubation period of pasteurellosis in experimental buffalo and bull calves was from three to six hours.
2. *Past. bovisseptica*, Indian strain, killed experimental buffalo calves in 32·7 hours (average) and a bull in 50·25 hours exhibiting a high degree of virulence.
3. *Pasteurella* invasion of blood circulation determined by different bacteriological methods was as follows:

In buffalo calves

By rabbit inoculation test : 13 hours (average) after infection or 19·33 hours (average) before death.

By cultural test : 17 hours (average) after infection or 15·4 hours before death.

By microscopic examination : 21 hours (average) after infection or 11·7 hours (average) before death.

TABLE III

Microscopic, cultural and animal inoculation tests into the blood of cattle and buffaloes infected with *Pasteurella*

Time of collection of blood after infection.	Buffalo calf, 10 months				Buffalo calf, 1 year				Buffalo calf, 2 years				Bull calf, 2½ years			
	Body temperature	Results of test by			Body temperature	Results of test by			Body temperature	Results of test by			Body temperature	Results of test by		
	°F.	Animal inoculation	Culture	Microscope	°F.	Animal inoculation	Culture	Microscope	°F.	Animal inoculation	Culture	Microscope	Animal inoculation	Culture	Microscope	
0 hour	99.2	—	—	—	99.0	—	—	—	100.2	—	—	—	101.0	—	—	—
3 "	100.0	—	—	—	100.8	—	—	—	121.6	—	—	—	104.6	—	—	—
6 "	102.2	—	—	—	104.8	—	—	—	108.8	—	—	—	105.6	—	—	—
9 "	103.2	+	—	—	107.6	+	—	—	104.8	—	—	—	108.8	—	—	—
12 "	103.8	+	—	—	104.8	+	+	—	106.2	—	—	—	104.0	—	—	—
15 "	102.0	+	+	—	104.2	+	+	+	107.2	—	—	—	104.0	—	—	—
18 "	100.4	—	+	—	104.6	—	+	+	106.0	—	—	—	104.6	—	—	—
21 "	99.8	—	+	+	102.6	—	+	+	106.6	—	—	—	108.8	—	—	—
24 "	99.2	—	+	+	101.6	—	+	+	104.2	+	+	—	105.2	—	—	—
27 "	97.2	—	+	+	104.4	—	+	+	106.4	+	+	+	108.2	—	—	—
30 "	Died at 2.40 p.m. on 26-11-1949	—	—	+	99.2	—	+	+	107.4	—	—	—	108.4	—	—	—
33 "	—	—	—	—	Died at 5.30 p.m. on 26-11-1948	—	—	—	106.0	—	—	—	108.4	—	—	—
36 "	—	—	—	—	—	—	—	—	104.5	—	—	—	104.6	—	—	—
39 "	—	—	—	—	—	—	—	—	Died at 1.0 a.m. on 26-11-1948	—	—	—	108.4	—	—	—
42 "	—	—	—	—	—	—	—	—	—	—	—	—	108.4	—	—	—
45 "	—	—	—	—	—	—	—	—	—	—	—	—	108.4	—	—	—
48 "	—	—	—	—	—	—	—	—	—	—	—	—	108.4	—	—	—

NOTES.—1. Plus and minus signs indicate respectively the positive and negative results of the tests actually carried out and where no tests are carried out the space is left blank. 2. Every animal developed at the seat of injection a big swelling which measured on the average 5 in. horizontally, 7 in. vertically and 1-6 in. in thickness. The swelling was hot and very painful. Sneakers from the swelling exudate showed *Pasteurella* in a rare number.

3. Heart blood culture made after the death of these animals gave rise to pure *Pasteurella* colonies after 24 hours incubation in buffalo calf No. 1 and after four days incubation in the case of the other animals.

4. The post-mortem examination reports of these animals are given in Figs. 1 to 4.

5. Post-mortem examination reports are stated in the protocols given in the appendix.

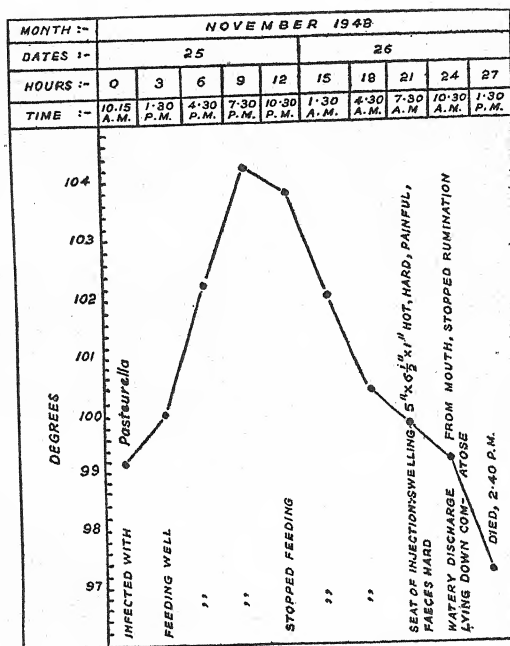


FIG. 1. Temperature chart of a ten-month old buffalo bull calf infected with pasteurellosis

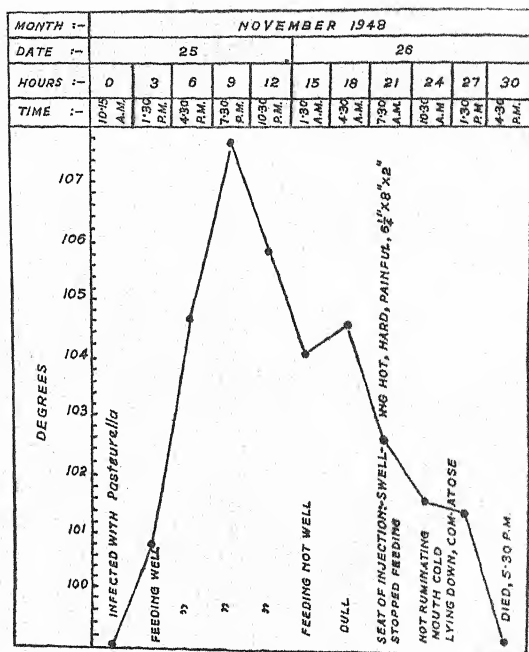


FIG. 2. Temperature chart of a one-year old Buffalo bull calf infected with pasteurallia

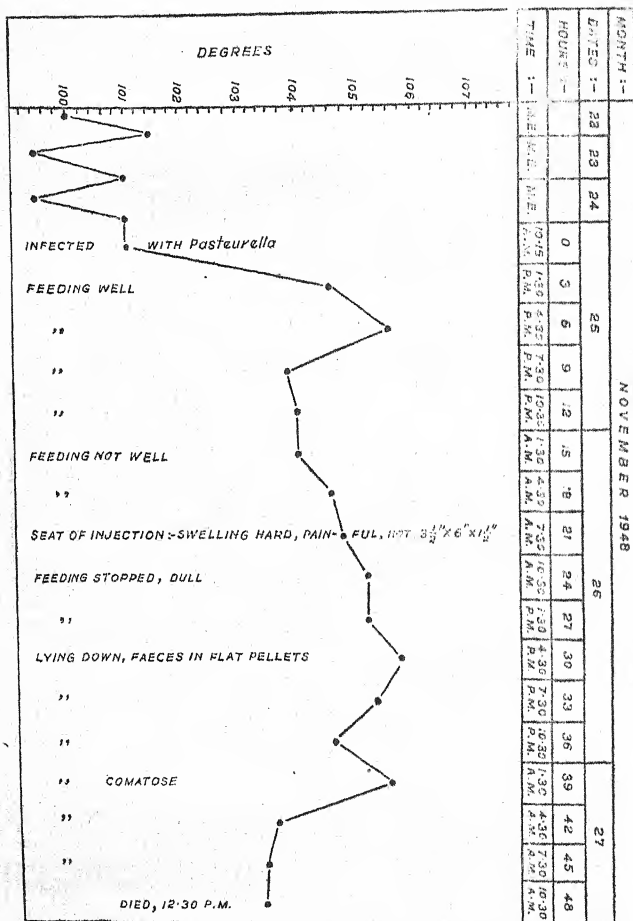


FIG. 4. Temperature chart of a two-year old bull calf infected with pasteurellosis

In bull calf

By rabbit inoculation test : 36 hours after infection or 14-51 hours before death.

By culture test : 42 hours after infection or 8-15 hours before death.

By microscopic examination : 45 hours after infection or 5-15 hours before death.

The above results proved that rabbit inoculation test carried out with the blood of affected animals afforded the best means of diagnosing pasteurellosis early, as it was the only test which was capable of detecting *Pasteurella* infection as soon as septicaemia had set in.

DISCUSSION

According to Hutyra and Marek [1946] the minimum incubation period in experimental inoculation is 6 hours in the bull and 12 hours in the buffalo ; but in the experiments conducted by the author it was three and six hours respectively.

It is stated already that previous workers had not extensively attempted the best method of diagnosis of pasteurellosis during life of the attacked animals. They have generally described what materials from a dead case should be collected and examined microscopically for the disease. But many workers must have experienced that smears collected from the heart blood and internal organs, of even normally dead animals in which putrefaction has set in, often have shown bipolar stained organisms. A laboratory diagnosis by the microscopic examination of blood smears which are collected from decomposed carcasses and which are not stained by Gram's stain for differential purposes is, therefore, inadvisable in pasteurellosis. Further in these days of scientific advancement, it is rather objectionable to ask the field workers to hold, for the purpose of collection of smears and other materials, a post-mortem examination in a case suspected to have died of any deadly contagious disease, such as anthrax, pasteurellosis, black quarter, etc. for field workers can do so only in the open grazing area in Indian villages ; the infection as a consequence becomes easily disseminated to other healthy animals directly or indirectly, either immediately or sometime later. In some provinces in India cutting open an anthrax carcase is prohibited by law ; it is, however, possible that Veterinary Officer suspecting pasteurellosis may open an anthrax carcase, if the present practice of collecting materials for microscopic confirmation from dead cases is not stopped as far as possible or discouraged. This can be done provided information is made available regarding the right materials to be collected during life and the best method of laboratory diagnosis. These were the main points considered during the enquiry, the results obtained have furnished the necessary information.

SUMMARY

It might be observed from the foregoing results that septicaemia actually occurred much earlier than what was believed to be so far in pasteurellosis. This septicaemia can be detected only by carrying out animal inoculation test in rabbits which are most susceptible to the disease. Other methods of detection, viz. cultural test and microscopic examination are not so efficient as the biological test. Diagnosis of

Pasteurella outbreaks in the field can be accurately made, when fresh dead cases are not available, by collecting a few c.c. of blood from an affected animal which is, in as advanced a stage of the disease as possible, i.e. about 20 and 14 hours before death in buffaloes and cattle respectively, and subjecting the blood or arranging to subject it to rabbit inoculation test and recovering the pathogenic organisms from the experimental rabbit. A young animal preferably a buffalo should be selected for the collection of blood if more than one cases are available.

The observation of the workers in the Western countries is that buffaloes are more susceptible to pasteurellosis than cattle. Indian field workers' experience has been also the same. But Bennet [1926] stated that both the species of animals in India were of approximately equal susceptibility. In the experiments reported in this article it has been revealed that the first invasion of *Pasteurella* occurred in blood circulation 2-15 hours, 13 hours and 36 hours after infection in the rabbit, buffalo and bull, respectively which indicates conclusively that the degree of susceptibility of each of the species mentioned to pasteurellosis is in the descending order and that the buffalo is more susceptible than the ox. The susceptibility of animals to a disease particularly of bacterial origin can be judged better by the data of the first invasion of the causal agent in the blood circulation and of the percentage death due to the same than by the latter alone.

ACKNOWLEDGEMENTS

This work was carried out under the Bacteriological Research Extension Scheme sanctioned by the Government of Bombay. Mr. D. T. Parnaik, G.B.V.C., Assistant Bacteriologist and Messrs. P. R. Dhake, G.B.V.C. and V. B. Kulkarni, G.B.V.C., Graduate Assistants, rendered invaluable help in carrying out the experiments. Grateful thanks are due to Mr. L. Sahai, M.Sc., M.R.C.V.S., Director of Animal Husbandry and Veterinary Science, Bombay Province and Mr. S. R. Chadha, B.Sc., M.R.C.V.S., Principal, Bombay Veterinary College, Bombay, for their encouragement in this work.

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APPENDIX

PROTOCOLS

Post-mortem examination report No. 1

Buffalo bull calf, age ten months, injected with *Past. bovisseptica* mixed culture of strain Nos. 216 and 226 on 25 November 1948 at 10-15 a.m., died at 2-40 p.m. on

26 November 1948. Post-mortem examination held on 26 November 1948 at 2-50 p.m.

Rigor mortis: Not set in, carcase in good condition.

Seat of injection: Gelatinous, thick exudate in large quantity.

Pharynx: Congested; Glottis: Congested; Trachea: Foamy mucous; Intercartilag space: Congested; Oesophagus: Normal; Pericardial sac: Nothing unusual; Ventricles: Endocarditis in the left ventricles; Lungs: Consolidated areas which sink halfway in water.

Kidney: Surface, cortex and medulla deeply congested; Urinary bladder: Empty; Liver: Surface and parenchyma, deeply congested, no liver flukes; Gall bladder: 150 c.c. bile; Spleen: Nothing unusual, Parenchyma slightly congested.

Abomasum: All the folds congested, pylorus deeply congested; Fundus: Deeply congested; Caecum: Parasitic nodules, deeply congested; Large intestine: Deeply congested with small balls of faeces wrapped in large quantity of gelatinous mucus; Mesenteric glands: Enlarged.

Post-mortem examination report No. 2

Buffalo bull calf, age one year, injected with *Past. bovis septica* mixed culture of strain Nos. 216 and 226 on 25 November 1948 at 10-20 a.m., died at 5-30 p.m. on 26 November 1948. Post-mortem examination held at 9-30 p.m.

Rigor mortis: Not set in.

Seat of injection: Gelatinous, thick exudate.

Pharynx: Congested, Larynx: Congested; Trachea: Congested; Intercartilag space: Congested; Lungs: Congested patches; Epicardial sac: Empty; Epicardium: Congested patches; Left ventricle: Endocarditis.

Spleen: Surface normal, Parenchyma congested; Liver: Liver flukes present in the bile ducts; Gall bladder: 200 c.c. bile; Kidney: Surface and parenchyma congested; Medulla: Swollen, gelatinous soft and yellow in colour; Urinary bladder: Full, about 200 c.c. urine.

Mesentery: Congested, engorged blood vessels; Abomasum, fundus and pylorus: Deeply congested; Small intestines: Congested; Caecum: Punctiform haemorrhages; Large intestine: Congested.

Post-mortem examination report No. 3

Buffalo bull calf, age two years, injected with *Past. bovis septica* mixed culture of strain Nos. 216 and 226 on 25 November 1948 at 10-15 a.m., died at 1 a.m. on 27 November 1948. Post-mortem examination held on 27 November 1948 at 7-0 a.m.

Rigor mortis: Not set in.

Seat of injection: Gelatinous fluid, deeply congested.

Pharynx: Congested; Larynx: Congested; Intercartilag space: Congested, foamy mucous in the trachea; Oesophagus: Superior part congested; Lungs: Congested and bluish deep patches; Epicardial sac: 100 c.c. sanguineous fluid, engorged blood vessels; Ventricles: Both congested.

Liver : Surface and parenchyma congested ; Gall bladder : 150 c.c. bile, m.m. : nothing unusual ; Kidney : Medulla, cortex congested, hylus yellowish white ; Urinary bladder : Empty, m.m. : congested ; Spleen : congested, parenchyma congested.

Fundus and pylorus : Deeply congested ; Small intestines : Punctiform haemorrhages ; Caecum : Deeply congested ; Rectum : Congested ; Large intestines : Congested throughout, straw coloured exudate in the abdominal cavity, congested blood vessels on the stomach.

Post-mortem examination report No. 4

Bull calf, age two half years, injected with *Past. bovisseptica* mixed culture of strain Nos. 216 and 226 on 25 November 1948 at 10.15 a.m., died on 27 November 1948 at 12.30 p.m. Post-mortem examination held on 27 November 1948 at 2.30 p.m.

Rigor mortis : Not set in.

Seat of injection : Gelatinous mass, deeply congested, engorged blood vessels.

Pharynx : Congested ; Larynx : Congested ; Intercartilag space : Congested ; Trachea : Congested ; foaming mucous on the floor of the trachea ; Lungs : Congested patches ; Oesophagus : Congested ; Epicardial sac : About 150 c.c. fluid ; Pericardium : Congested, blood vessels engorged ; Ventricles : Deep dark patches (congested).

Liver : Congested ; Gall bladder : Dark bile about 200 c.c., m.m. : nothing unusual ; Kidney : Surface congested, Cortex and medulla congested, Urinary bladder : Empty, m.m. congested ; Spleen : Congested patches, parenchyma congested.

Fundus and Pylorus : Deeply congested ; Small intestines : Punctiform haemorrhages ; Caecum : Punctiform haemorrhages ; Rectum : Deeply congested patches ; Large intestines : Congested, blood vessels on the alimentary canal congested.

SOME OBSERVATIONS ON MORTALITY IN GOATS

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(Received for publication on 3 September 1949)

(With one text-figure)

THERE are no reliable data available on the mortality among goats in India. The success or otherwise of a breeder depends upon the rate of mortality in his stock, high mortality being a constant worry to him, as it not only retards progress but seriously affects his returns also. Therefore, to throw some light on this problem some aspects of mortality in goats have been discussed. The present contribution which is a compilation of data on mortality of Betal flock bred at Hissar, of Barbari goats kept at the Mission Poultry and Goat Breeding Farm, Etah, Jannapari and some other Cross breeds (Toggenberg and desi) and Hill goats maintained at Hissar Farm. Much, as we would have liked to include the data of mortality of goats in villages under varying conditions of climate, we have not been able to do so, mainly owing to the inavailability of data. The two places, Hissar and Etah, where these flocks of goats have been maintained, possess dry climate. Though, there is considerable difference in the yearly rainfall at these places; it is 15 in. per annum at Hissar and 24-28 in. at Etah.

MANAGEMENT

The main flocks, under consideration are those of Jannapari, Betal and Barbari breeds which have been maintained with a view to improve the milk yield of these breeds by selective breeding. The goats go for grazing on the farm *bir*; and the bucks and milk goats get concentrates in addition to grazing. The goats are housed in free ventilated sheds, amply protected from extremes of heat, cold and rains. As a safeguard against parasites, the goats are drenched with one per cent solution of copper sulphate and mustard every month and occasionally dipped to protect against external parasites. The sick ones are isolated and tended to in the hospital attached to the farm, where they are treated, after a proper diagnosis has been made.

MORTALITY IN VARIOUS BREEDS

Table I gives the average of mortality in the various breeds. The data covers the period from 1928 to 1944 in case of Betals, from 1931 to 1941 in Jannaparis, from 1931 to 1944 in Barbaris, from 1931 to 1936 in Toggenbergs, and from 1941 to 1944 in Hill goats,

TABLE I

Mortality in various breeds

Serial number	Breed	Adult		Average	Kids		Average	Remarks
		Male Per cent	Female Per cent		Male Per cent	Female Per cent		
1	Betal	10.6	10.8	10.7	29.5	29.5	29.5	Adults above 1½ years
2	Jamnnapari	8.9	9.9	9.4	28.6	14.7	21.6	Kids from birth to 1½ years
3	Barbari	4.8	13.0	8.6	16.4	16.1	16.2	Average of 9 years
4	Toggenberg	23.3	65.8	44.5	66.6	29.7	47.9	Average of 4 years
5	Cross-bred	0.0	4.2	4.2	41.8	18.0	29.8	Average of 4 years
6	Desi	..	9.5	9.5
7	Hill goats	0.0	3.3	3.3	14.8	11.8	13.3	Average of 3 years

It is observed, that mortality, as expected, is higher in the kids than in the adults. With the exception of Toggenbergs, for which only meagre data were available, the mortality in Betal was higher than that of the other breeds; it was the lowest in the Hill goats. There was no variation in mortality on account of the sex difference in Betals, but in Jamnapari and Barbari, the mortality was higher in the male kids than in the female ones. The mortality varies from year to year in all the breeds (Appendix I), the variations being most prominent in the adult stock. The mortality was not much higher amongst the adults of Betal breed during 1939-42, which were famine years.

It is observed that the mortality is higher in heavy milking breeds, as borne out by Table II, which gives comparative data of milk yield and mortality.

TABLE II

Comparative data of milk yield and mortality

Serial number	Breed	Average mortality		Average milk yield per lactation	Fat per cent	Days in milk	Milk performance		
		Adult female goats	Kids				Days dry	Maximum yield	Daily average yield
1	Betal	10.8	20.5	489.8 lb.	4.5	224	106	3.8	2.6
2	Jamnnapari	9.9	21.6	465.6 "	5.3	237	104	3.0	1.5
3	Barbari	13.0	16.2	381.0 "	3.8	252	147	3.2	1.3
4	Desi	9.5	..	446.1 "	4.6	241	98	3.5	1.8
5	Cross-bred	4.2	29.8	898.2 "	4.7	359	115	4.0	2.5
6	Hill goats	3.3	13.3	8 to 12 ozs.

Seasonal variations in mortality

A year has been provisionally divided into four quarters starting from April. The first quarter including April, May and June is hot, dry summer, the second from July to September is hot, humid and rainy, the third from October to December and the fourth from January to March are winter months. One of the objects of this seasonal division was to find out the best kidding season by a study of mortality in each season. The usual dry season at Hissar starts towards the end of March and continues to the end of June, followed in the beginning of July by the monsoon period. The hot humid weather lasts till the end of September, when, from October, the winter may be said to set in. The temperature is low in December, January and February. The average rainfall at Hissar is about 17 in. (15 in. during the years under review), but failure of rains is common, when scarcity conditions prevail. The rains usually continue during the monsoon months, when abundant grazing and browsing in the forest, attached to the farm, is available, but much depends on the distribution of rainfall in the different months. The winter rains are usually meagre and uncertain and have uneven distribution. During the last quarter, i.e. January to March, there is usually scanty feeding and there is shedding of tree leaves, which may have profound effect on the health of goats in that quarter.

It is observed from Tables III and IV that the average mortality on the whole is the least in the second quarter and the highest in the fourth, in both the males and females. Thus comparing the casualties in summer and winter months, it is seen that mortality in winter months is almost double than that in the summer months.

TABLE III

Mortality in summer and winter

Quarter		Number of deaths	Percentage
1st	Summer	264	22.8
2nd		167	14.4
3rd	Winter	303	26.1
4th		424	36.4

Same is the case in Jamnapari, except that in male kids mortality is the least in the first quarter instead of in the second. In Barbari kids results are entirely different; in the female kids the highest mortality occurs in the first quarter and the lowest in the third. In the male kids, mortality is equally distributed throughout the year. Cross-breds behave exactly like the Betal kids, while in the Hill goats the male kids show equal mortality in summer and winter, while the females suffer more in summer than in winter. This is, perhaps, because the females cannot stand summer so well, as the males.

It is also noted that the mortality rate is not more in some quarters because of the higher birth rate in those, but, is due to seasonal changes (Table V).

In case of adults, however, season does not seem to have much influence on mortality as deaths are fairly equally distributed in all the four quarters except in the first one, in which mortality is only slightly less.

Monthly distribution of mortality

When monthwise distribution is taken into account, it is noted that the least number of deaths in the adult stock occur in the month of July, and in the kids in August. With the onset of summer, there occurs a decrease in mortality as shown below, till the lowest figure is attained in the month of August.

April	2.8	Per cent
May	2.2	do.
June	1.4	do.
July	1.5	do.
August	1.4	do.

In the winter period, more deaths occur in the post-winter months than in the pre- and mid-winter. On account of different distribution of mortality in the kids and adults, no particular month or months can be singled out in which there is low mortality both in the adults and kids.

Age wise distribution of mortality

Table VI summarizes the distribution of average mortality according to age in Betal goats from 1928-44 and in the Jamnapari and Barbari from 1931-44.

It is apparent that 18.6 per cent out of the 29.5 per cent mortality in the kids occurs from birth to the second week of life. After the age of four weeks (one month) there is a reduction in mortality and thereafter up to $1\frac{1}{2}$ years, the percentage of mortality is low. It may be stated that kids are weaned at 3-4 months of age. Again in the adult stock, at the age of two years, there is an increase in the percentage of mortality, since 3.7 per cent out of 9.7 per cent average mortality occurs at this age. This is also high as compared with 1.7 mortality at $1\frac{1}{2}$ years of age. This is most probably due to the strain of first kidding upon female goats, which generally kid for the first time at this age. This is borne out by the fact that about 46.9 per cent of the deaths (see Table VII) which occur at this stage, are due to uterine and udder affections.

TABLE V
Comparison of rates of kidding and mortality

Breed Quarter		Mortality per cent						Kidding per cent						Remarks			
		Jannapuri		Barbari		Cross-bred		Hill goats		Jannapuri		Barbari			Cross-bred		
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female		Male	Female	Average
1st	24	28	6.1	3.9	12.6	6.1	4.2	4.8	13.2	14.6	13.4	16.5	12.5	10.0	17.2	13.6	Data being meagre Hill goats not given
2nd	5.2	2.4	2.5	4.0	0.7	1.2	3.2	2.5	4.1	3.9	13.8	12.3	13.0	
3rd	9.3	4.1	2.2	4.3	6.8	2.0	4.4	3.6	62.2	61.5	56.9	52.4	37.1	41.6	41.6	37.2	
4th	11.0	5.2	5.6	3.3	21.5	3.3	2.7	0.9	19.2	10.4	19.8	11.7	13.1	43.0	43.0	49.4	

TABLE VI
Mortality in different age groups

[illegible]

TABLE VII

Causes of mortality in the female

	Peritonitis	Rupture intestine	Septic metritis	Rupture uterus	Mastitis	Lung affection	T. B.	Septicæmia	Liver affection
Number died	2	2	16	10	11	13	7	4	7
Percentage	2.5	2.5	20.3	13.8	13.8	18.5	8.8	5.0	8.8

After the goats are two years old, there is a decided fall in the record of mortality and during the succeeding years of the goat's life, the mortality figures remain fairly low and uniform.

Similarly in the Jamnapari kids, there is higher percentage of mortality up to the age of one month; 6.5 per cent out of a percentage of 21.6 deaths occur at this stage. From the age of four months onwards, there is a decided fall. In the adult female goats there is more mortality at the age of 2-3 years than at any other age. (Quite like Jamnapari, the Betal and Barbari kids suffer high mortality from birth up to one month of age; from the age of three months there is a decided fall up to 1½ years. In the adult female goats out of 13 per cent mortality, 6.3 per cent is distributed at the age of 2-3 years.

Effect of rainfall, temperature and humidity

In order to find out whether mortality is influenced by climatic factors like maximum and minimum temperatures, humidity and rainfall, the data were reviewed from the statistical point of view. Correlation co-efficients between mortality and various climatic factors collectively for all the years and also for the different quarters were worked out with the help of the formula, $\frac{S(xy)}{S(x)^2 S(y)^2}$. This was done for the Betal breed only as full data for other breeds were not available. Table VIII shows the result of the analysis.

From the analysis it appears that the separate atmospheric factors, when compared statistically, have got very negligible effect on mortality; although the data, as plotted in a graph (Fig. 1), without subjecting them to statistical analysis appear to indicate that there is some relationship between mortality and rainfall. From Fig. 1, it is seen that there is high mortality, especially in kids, in the years following drought years. For instance, in the years 1933 and 1934 following dry years, and in 1939-42, which are years of low rainfall, there is increased mortality.

TABLE VIII
Relationship between mortality, temperature, humidity and rainfall in different quarters

Items compared	Value of correlation coefficient									
	Adults			Kids			Quarters			
	Whether significant or not		Whether significant or not $P = 0.05$	Whether significant or not		Whether significant or not $P = 0.05$	Whether significant or not			
	Male	Female		Male	Female		1st	2nd	3rd	4th
Mortality x maximum temperature	+0.7810	-0.3853	Insignificant $P = 0.05$	-0.143	-0.175	Insignificant $P = 0.05$	-0.1773	-0.0742	-0.1438	+0.0645
Mortality x minimum temperature	+0.5637	-0.1880	do	+0.0825	-0.3341	do	-0.0161	+0.1928	+0.0004	+0.0270
Mortality x humidity	-0.0237	-0.1386	do	+0.0000	-0.0115	do	-0.2032	+0.0217	+0.0092	0.1543
Mortality x rainfall	-0.2090	+0.1592	do	+0.1206	-0.0373	do	+0.1786	+0.0213	+0.1923	0.0203

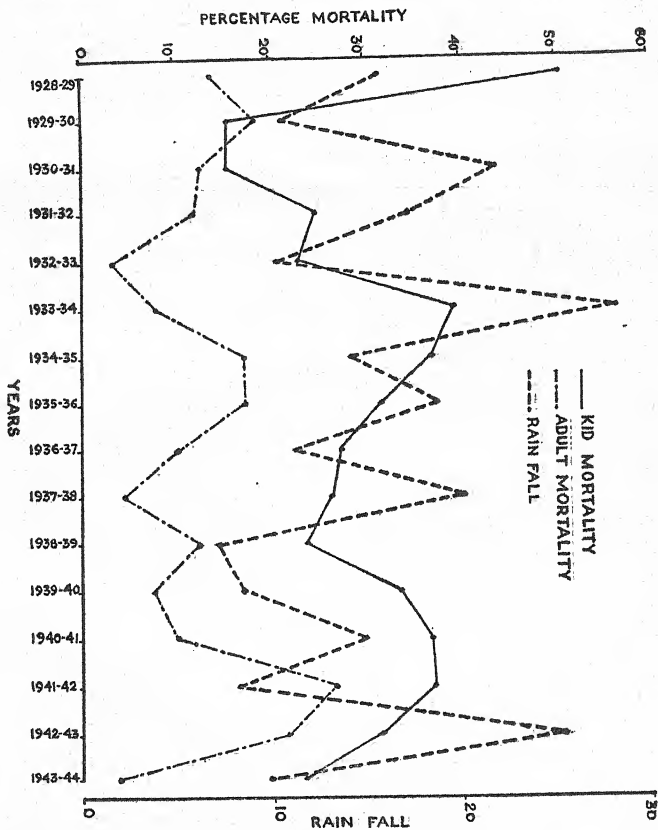


FIG. 1. Mortality among kids and adult goats (Betal) in relation to rainfall (1928-1944)

E

Diseases causing mortality

The data in this connection are available for Betal goats only. These can be classified as indicated below :

Serial number	Diseased conditions	Percentage of mortality	
		Adults	Kids
1	Digestive tract	8.6	38.6
2	Respiratory tract	20.1	31.1
3	Liver	3.8	5.5
4	Kidneys	0.8	0.5
5	Brain	1.2	0.9
6	Udder (mastitis—female goats)	18.0	..
7	Uterus (metritis and other uterine affections—female goats)	30.0	..
8	Navel	..	0.0
9	Debility	..	3.3
10	Premature births	..	11.4
11	Congenital deformity	..	0.3
12	Killed by wild animals	1.6	2.7
13	Goat pox	1.2	..
14	Tuberculosis	7.5	0.3
15	Foot and mouth	..	0.5
16	Tetanus	0.4	..
17	Aptha	0.0	0.4
18	Miscellaneous	7.1	3.1

It is seen that the diseases of the digestive system are the most important cause of death followed by respiratory and uterine diseases. The digestive and respiratory troubles are responsible for heavier mortality in kids, while the uterine maladies cause deaths in adults. It is a noteworthy fact, that the parasitic diseases, which are known to cause heavy mortality in village goats, appear to be altogether absent as a cause of mortality at Hissar; primarily because animals are regularly drenched and secondly because the area is dry, there is low incidence of parasites. It may also be stated here that brucellosis has existed in this herd and most of the uterine troubles can be attributed to that; udder troubles have been traced to an outbreak of mastitis caused by contagious agalactia organism.

DISCUSSION

The data regarding mortality among the Betal goats for the last 16 years at Hissar is indicative of the fact that deaths due to various diseased conditions are not so high in the adult goats (average 10.7 per cent) as in the young ones (29.5 per cent); in the latter mortality is $2\frac{1}{2}$ times more than in the former. The comparative data over a number of years for Hill goats kept at Hissar, Jamnapari (10 years), Barbari (13 years), Toggenburg (5 years) and Cross-bred (5 years) goats kept at Etah Farm, U. P., under similar management, gives similar findings. It is also found that mortality in the Betal goats kept at the Hissar is somewhat higher than the other breeds. Besides, there is higher mortality in the milch breeds of goats than in the non-milch breeds. Further, in the milch breeds there appears to be some relation between milk yield and mortality, for it is found that goats of higher milk yield show more mortality than goats of lower milk yield. The seasonal distribution of mortality in the Betal goats varies a great deal in the kids, for it is found that in the kids mortality is the highest in the fourth quarter (January to March) and the lowest in the second quarter (July-August-September). Considered on summer and winter basis, it is found that nearly double the mortality is recorded in the winter months than in the summer ones. The mortality in the adult stock is fairly uniformly distributed in all the four quarters. In this respect, the Jamnapari goats (allied to Betal) behave exactly like Betal, although kept at a different place. The Barbari breed of goats does not behave exactly like the other two, as in this breed the mortality is equally distributed in the summer and winter seasons. However, the atmospheric conditions as such, on statistical analysis, appear to have very little influence on mortality. Kidding percentage during the different months does not appear to be co-related with mortality. It, therefore, appears likely that nutrition may have some influence on mortality, especially in kids, for it is in the fourth quarter, that there is found to be a great change in the composition of the leaves of trees which are generally eaten by goats. The percentage of dry matter in the leaves is the highest, and the percentage of crude protein and phosphate the lowest in this quarter [Ray and Momin, 1943]. The change in the composition of the fodder of the goats is likely to effect the nutritional value of goat's milk, thereby affecting adversely the health of the suckling kids.

SUMMARY

Data regarding mortality during the past 16 years in Betal goats kept at Hissar are presented in this paper; comparative data on mortality of other breeds of goats like Hill goats, Jamnapari, Barbari, Toggenburg and Cross-bred, are also given. The results are :-

Breed	Mortality percentage	
	Adults	Kids
Betal	10.7	29.5
Jamnapari	9.4	21.6
Barbari	8.6	16.2
Toggenburg (foreign breed)	44.5	47.0
Cross-bred	4.2	29.8
Hill goats	3.3	13.3

It has been noted that in the kids mortality is higher in winter months than in summer, and is more in milch breeds than in others. Digestive and respiratory diseases are the common cause of mortality in the kids and uterine trouble in goats.

ACKNOWLEDGEMENT

We are under a sense of gratitude to Shri P. N. Nanda, M.R.C.V.S., formerly Superintendent, Government Livestock Farm, Hissar, for his guidance and valuable suggestions in the preparation of this paper. S. Tirlok Singh, M.Sc., Research Assistant of Hissar Farm is to be thanked for his help in statistical analysis.

APPENDIX I

Showing mortality amongst Beetal breed of goats at Hissar farm from 1 April 1928 to 31 March 1944

	Adults			Kids		
	Number present	Number died	Percentage of mortality	Number present	Number died	Percentage of mortality
Male	162	14	8.6	3,774	1,158	30.6
Female	1,816	239	13.1			
Total	1,978	253	12.8			

Serial number	Year	Average number present	Number died	Percentage of mortality	Average number present	Average number died	Percentage of mortality
1	1928-29	37	5	13.5	107	54	50.5
2	1929-30	61	12	19.7	134	21	15.7
3	1930-31	74	9	12.2	124	19	15.3
4	1931-32	79	9	11.4	176	44	25.0
5	1932-33	82	3	3.7	221	51	23.1
6	1933-34	98	8	8.2	220	87	39.5
7	1934-35	111	19	17.1	238	88	37.0
8	1935-36	105	18	17.1	168	53	31.5
9	1936-37	110	11	10.0	218	60	27.5
10	1937-38	121	6	5.0	243	65	26.5
11	1938-39	119	14	11.8	231	55	23.8

APPENDIX I—*contd.*

Serial number	Year	Average number present	Number died	Percentage of mortality	Average number present	Average number died	Percentage of mortality
12	1939-40	168	13	7.7	327	109	33.3
13	1940-41	218	22	10.1	362	133	36.7
14	1941-42	206	53	25.7	410	156	38.0
15	1942-43	197	43	21.8	315	98	31.1
16	1943-44	192	8	4.2	280	65	23.2
	<i>Total</i>	1978	253	19.2	3774	1158	473.2

Mean=12.5
 Standard deviation=6.53
 Standard error of mean= ± 1.63
 Coefficient variation=52.24

Mean=29.6
 Standard deviation=14.75
 Standard error= ± 3.69
 Coefficient of variation=49.40

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STUDIES ON THE BIOLOGICAL VALUE OF PROTEINS OF SOME COMMON FEEDS

I. BIOLOGICAL VALUE OF THE PROTEINS OF WHEAT STRAW AND SOME OILCAKES

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IN formulating human diets it has been customary to differentiate between proteins of different classes of foodstuffs by experimentation on rats. In the feeding standards for cattle and other ruminants, however, proteins of different feeding-stuffs have been assumed to be substantially equal in nutritive value. Until recently, no serious attempt had been made to study the qualitative difference in the proteins of animal feeds. This is mainly due to the technical difficulties and high cost involved in carrying out the necessary experiments with ruminants. On the other hand, if small laboratory animals (e.g. rats or rabbits) are used, it is not possible to confirm whether the results obtained with them can be applied to ruminants without parallel experiments being conducted on the latter. The use of a non-ruminant for this purpose is further complicated by the widely different capacities of the two types of animals in utilizing coarse fodders.

In view of the paucity of accurate information regarding the nutritive value of the proteins of cattle feeds and the immense practical importance of the problem, a comprehensive study of the subject was initiated. Of the methods employed for estimating the biological value of proteins, the Thomas-Mitchell method [Mitchell, 1924] has been most extensively used in experiments on non-ruminants. According to this method, the biological value (B.V.) of a protein for maintenance is given by :

$$B.V. = \frac{I_N - (F_N - M_N) - (U_N - E_N)}{I_N - (F_N - M_N)} \times 100$$

where, I_N =nitrogen intake, F_N =total faecal nitrogen, M_N =metabolic faecal nitrogen, U_N =total urinary nitrogen and E_N =endogenous urinary nitrogen.

The above formula shows that for the estimation of the biological value, the metabolic faecal nitrogen and endogenous urinary nitrogen data of the experimental subjects are essential pre-requisites. The metabolic faecal nitrogen data are also required for the calculation of the true digestibility coefficient (T.D.C.), since it is given by :

$$T.D.C. = \frac{I_N - (F_N - M_N)}{I_N} \times 100$$

Studies on these pre-requisites have recently been reported by Kehar *et al.* [1943] and Mukherjee and Kehar [1949 a,b and c]. These have made it possible to investigate

the biological value and digestibility of the proteins of typical combinations of cattle feeds, regarding which no information is available. This article deals with the protein value of the combinations of wheat straw and some common oilcakes.

EXPERIMENTAL

The general procedure of conducting metabolism trials and the methods of analysis followed in the present investigation are practically the same as those described by Kehar *et al.* [1943] and Mukherjee and Kehar [1949 a]. The adjustment and collection periods for the metabolism experiments were 15 days and 10 days respectively. Intervening periods of 15 days or more were allowed between two consecutive experiments, during which a good stock diet was fed.

In these studies, the biological value and true digestibility of two or more concentrates or rations have been compared either (a) with the same animals in different periods or (b) with two or more identical groups of animals in the same period. Since the biological value varies with the percentage of protein in the ration [Mitchell and Hamilton, 1929], the protein level in the individual experiments was kept within narrow limits by controlling the roughage intake. Whenever any animal in a group or experiment grossly failed to satisfy the required conditions of uniformity in protein level and energy intake, observations made on it were omitted from the tables. In all cases, adult healthy animals were used and the biological values reported in this series of investigations refer to those for maintenance.

The biological value and true digestibility figures were calculated according to the formulae already given. The figure for endogenous urinary nitrogen was taken to be 0.02 gm. per kg. body weight for cattle [Kehar *et al.*, 1943]. Metabolic nitrogen was estimated graphically [Mukherjee and Kehar, 1949 c].

The experimental rations consisted of wheat straw and a supplement of one of the following nitrogenous concentrates at 9 and 6 per cent protein level. Preliminary experiments showed that wheat straw alone failed to maintain animals in nitrogen balance, hence the biological value data of the supplemented ration only are presented here. Of the three concentrate mixtures used, the first one consisted of groundnut cake (94 per cent), calcium carbonate (3 per cent), and sodium chloride (3 per cent); the second and third mixtures were similar to the first but contained colza cake and mustard cake instead of groundnut cake. The amount of food offered to the experimental animals was calculated according to the 0.73 power of their respective body weight, since the energy requirement of animals varied accordingly [Brody *et al.*, 1934]. Every attempt was made to keep the energy intake at a uniform level, viz. 4 lb. of starch equivalent per 1000 lb. body weight. Sen [1949] has compiled the data of some Indian experiments, which suggest that the energy requirement of Indian cattle is about 4 lb. of starch equivalent per 1000 lb. body weight for maintenance.

A comparative study on three types of cattle consuming the same ration was made so as to find out the possible effect of breed characteristics on the biological value and also if the inclusion of different types of animals in the same group affects the value as compared with those using a single type of animals.

RESULTS AND DISCUSSION

(a) *Experiment 1.—Observations on rations containing 9 per cent protein*

The nitrogen metabolism data of three bullocks fed on two similar rations containing wheat straw together with (i) groundnut cake mixture and (ii) colza cake mixture at 9 per cent protein level in two consecutive metabolism experiments are given in Table I.

TABLE I

Biological value and digestibility of the proteins of two oilcakes plus wheat straw in cattle at 9 per cent protein level.

(Metabolism data on daily basis.)

Bullock number.	Average body weight = W (lb.)	Food dry matter intake (gm.)	Food N		Faecal dry matter = F.D. (gm.)	F.D. ——— 0.73 W	Nutrient intake@ (per 1000 lb. body weight)	
			Roughage (gm.)	Concentrate (gm.)			Starch equivalent* (lb.)	Digestible protein (lb.)
Groundnut cake ration								
Ha 1	800	5339	22.2	46.1	2200	16.7	4.3	0.71
L 5	840	4342	17.4	48.1	2130	15.6	3.6	0.64
Ha 8	1128	7072	29.3	63.5	2860	16.9	4.4	0.73
Average	923						4.1	
Colza cake ration								
Ha 1	788	4279	16.2	47.5	2360	18.2	4.0	0.62
L 5	816	4211	15.6	50.4	2260	16.9	3.9	0.63
Ha 8	1088	6522	25.1	66.6	2720	16.5	4.6	0.87
Average	897						4.2	

* Assuming the starch value of the dry matter of the straw and cakes to be 25 and 77 per cent respectively.

@ Calculated according to the 0.73 power of the body weight.

TABLE I—*contd.*

Bullock number	Food N (Total) (gm.)	Faecal N		Urinary N		N balance (gm.)	Biological value per cent	Digestibility coefficient	
		Total (gm.)	Metabolic (gm.)	Total (gm.)	Endogenous (gm.)			True	Apparent

Groundnut cake ration

Ha 1	68.3	24.6	16.8	42.3	7.3	+1.4	42.1	88.6	64.0
L 5	65.5	24.8	17.4	41.4	7.6	—0.7	41.8	88.7	62.1
Ha 8	92.8	34.8	22.0	58.2	10.2	—0.3	39.0	86.2	62.5
Average						+0.1	41.3	87.8	62.9

Colza cake ration

Ha 1	63.7	26.1	17.9	33.5	7.2	+4.1	52.6	87.1	59.0
L 5	66.0	26.9	18.1	38.3	7.4	+0.8	46.0	86.7	59.2
Ha 8	91.7	32.0	21.0	53.3	9.9	+6.4	46.2	88.0	65.1
Average						+3.8	48.3	87.3	61.1

The data show that the total nitrogen and net energy intake of the animal was comparable on both rations. The average biological values of the proteins of the above mentioned combinations of feeds were 41.3 per cent and 48.3 per cent respectively; the difference was statistically significant. As can be expected from the biological value results, the nitrogen balance was also higher on the colza cake ration. The true digestibility coefficients of the proteins from groundnut and colza cake rations were 87.8 per cent, 87.3 per cent respectively.

(b) Experiment 2. Observations on rations containing six per cent protein

In this experiment the same two bullocks were fed, at different periods, concentrate mixtures containing groundnut, colza and mustard cakes along with wheat straw, the protein content of the three rations being about 6 per cent.

TABLE II

Biological value and digestibility coefficient of the proteins of three oilcakes wheat straw in cattle at 6 per cent protein level.
(Metabolism data on daily basis)

Bullock number	Average body weight = W (lb.)	Food dry matter intake (gm.)	Food N		Faecal dry matter = F.D. (gm.)	F. D. G-73 W	Nutrient intake @ per 1000 lb. body weight	
			Roughage (gm.)	Concentrate (gm.)			Starch equivalent* (lb.)	Digestible protein (lb.)
Groundnut cake ration								
Ha 12	1008	7109	31.5	30.7	31.80	20.4	4.3	0.44
Ha 13	984	6566	29.1	27.9	29.60	19.3	4.1	0.33
Coleza cake ration								
Ha 12	980	6173	26.5	30.4	2800	18.4	4.1	0.38
Ha 13	954	5712	24.4	29.5	2720	18.2	3.9	0.36
Mustard cake ration								
Ha 12	1120	6758	29.3	33.7	3160	18.8	4.0	0.35
Ha 13	1044	7155	31.3	30.0	3360	21.0	4.3	0.36

Bullock number	Food N (total) (gm.)	Faecal N		Urinary N		N balance (gm.)	Biological value (per cent)	Digestibility coefficient	
		Total (gm.)	Metabolic (gm.)	Total (gm.)	Endogenous (gm.)			True	Apparent
Groundnut cake ration									
Ha 12	62.2	30.5	23.6	31.0	9.2	+0.7	66.6	88.9	51.0
Ha 13	57.0	33.3	22.2	28.2	8.9	-4.5	59.0	80.5	41.6
Average							59.3	84.7	
Coleza cake ration									
Ha 12	56.9	30.0	21.2	26.0	8.9	+0.9	64.5	84.5	47.3
Ha 13	53.9	28.4	20.6	24.1	8.7	+1.4	66.6	85.5	47.3
Average							65.6	85.0	
Mustard cake ration									
Ha 12	63.0	35.1	23.8	30.1	10.2	-2.2	61.5	82.1	44.3
Ha 13	61.3	34.6	24.8	24.4	9.5	+2.3	71.1	84.0	43.6
Average							66.3	83.0	

* Vide footnote of Table I

The results show (*vide* Table II) that although the average biological values of the colza and mustard cake rations (65.6 per cent and 66.3 per cent respectively) were higher than that of the groundnut cake diet (59.3 per cent), the difference was not statistically significant. It seems likely that if the number of animals were larger, the difference might have been found to be statistically significant. The digestibility coefficients (84.7 per cent, 85.0 per cent and 83.0 per cent) of the nitrogen content of the three rations were, however, similar.

(c) *Experiment 3. Comparison of the biological value results on different breeds of cattle*

The results of this experiment are presented in Table III.

TABLE III

Biological value of the proteins of a ration composed of wheat straw and mustard cake on two breeds of cattle

(Metabolism data on daily basis)

Bullock number	Average body weight (lb.)	Starch equivalent per 1000* lb. body weight (lb.)	Food N (Total) (gm.)	Faecal N (Total) (gm.)	Urinary N		Biological value (per cent)
					Total (gm.)	Endogenous (gm.)	
On local bullocks (Ration : 5.1 per cent C.P.)							
L 4	501	3.5	25.3	16.3	11.0	4.5	71.0
L 5	516	4.3	33.7	18.8	14.5	4.7	67.7
Average							69.3
On Kumaun bullocks (Ration : 5.0 per cent C.P.)							
K 1	315	4.0	21.9	12.5	8.7	2.9	71.0
K 4	340	4.4	23.2	13.8	9.5	3.1	70.0
Average							70.5

* *Vide* footnote of Table I

As in other Tables, the data of only those pairs of animals have been recorded, whose food intakes have been fairly comparable. The biological value figures (69.3, 70.5) show that there is hardly any difference in the efficiency of utilization of absorbed nitrogen by the two types of cattle, namely local and Kumaun cattle. It is interesting to note that a similar value (66.3 per cent) was obtained for the biological value of an identical ration on Harijana bullocks in Experiment 2 (*vide* Table II).

(d) *Comparison with published results*

It is difficult to compare our observations with those recorded in the literature. So far as we are aware, there are hardly half a dozen published articles reporting the biological evaluation of feed proteins on cattle [Morris and Wright, 1933 a, 1933 b, 1935; Morris *et al.*, 1936; Morris and Ray, 1939; Harris *et al.*, 1943]; of these, only two [Morris and Wright, 1935 and Harris *et al.*, 1943] are concerned with the biological value for maintenance.

Comparatively more work has been reported on sheep [Turk *et al.*, 1934, 1935; Miller *et al.*, 1937, 1939; Smuts and Marais, 1939 a, 1939 b, 1940 a, 1940 b; Harris and Mitchell, 1941 a, 1941 b; Johnson *et al.*, 1942; Ferguson and Neave, 1943]. None of the articles, however, deal with rations similar to those used in the present investigation.

Turk *et al.* [1934] reported the biological value of the proteins of clover hay and alfalfa hay, with or without the addition of corn, to be about 80 per cent on growing lambs at 10 per cent protein level. Swanson and Herman [1943] give the corresponding values of the proteins of alfalfa hay and milk for growing heifers as 75 per cent and 67 per cent respectively. But the biological value of the mixed proteins (at 9 per cent level) of wheat straw and groundnut cake (41 per cent) or that of the colza cake combination (48 per cent) do not necessarily indicate a comparatively poor quality of the proteins of these rations, since the experimental conditions in the present investigation were different from those mentioned above. Our figure of about 66 per cent for the biological value of the wheat straw plus colza cake and of the wheat straw plus mustard cake rations at 6 per cent protein level can, however, be compared with the value of 79 per cent for the biological value of the casein ration fed to sheep at maintenance level by Harris and Mitchell [1941 a]. From this comparison, it may be argued that the colza and mustard cake rations furnished poorer assortment of amino acids than casein. The groundnut cake combinations have consistently been shown to be poorer than the corresponding colza or mustard cake ration in the present investigation. While no information about the biological value of the latter two cakes is available, the biological value of groundnut cake protein for young rats has been reported by some workers. The recorded values are: 58 per cent at 8 per cent level [Mitchell *et al.*, 1936]; 57 per cent at 10 per cent level [Swaminathan, 1937] and 72 per cent at 9 per cent level [Smuts and Malan, 1938].

SUMMARY

The biological values and the true digestibility coefficients of the proteins of certain rations composed of wheat straw and three important oilcakes were determined on adult cattle.

The biological value results were:

(a) At 9 per cent protein level:

Wheat straw (W. S.) plus groundnut cake (G.N.C.), 41.3 per cent; W.S. plus colza cake (C.C.), 48.3 per cent. The difference between the two figures was statistically significant.

(b) At 6 per cent protein level:

W.S. plus G.N.C., 59.3 per cent; W.S. plus C.C., 65.6 per cent; W.S. plus mustard cake, 66.3 per cent. Statistically the groundnut cake combination had the lowest value.

The average true digestibility coefficients of the proteins of the first two rations were respectively 87.8 per cent and 87.3 per cent, while those of the last three diets were 84.7 per cent, 85.0 per cent and 83.0 per cent. These results indicate that, at a given protein level, there was no significant difference between the rations.

A comparison of a limited number of data suggested that the estimate of the biological value was not affected measurably by the type or breed of cattle used.

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STUDIES ON THE BIOLOGICAL VALUE OF PROTEINS OF SOME COMMON FEEDS

II. BIOLOGICAL VALUE OF THE PROTEINS OF PADDY AND OAT STRAWS IN COMBINATION WITH SOME COMMON OIL CAKES

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SINCE paddy straw is extensively used as roughage in India, investigations were undertaken to find out the biological value of the protein content of rations consisting of paddy straw and some common oil cakes namely, colza and mustard. Unlike other straws, oat straw is relished by sheep as well as cattle, hence observations were made on oat straw in combination with groundnut and colza cakes. In the oat straw experiment, parallel observations were simultaneously made on sheep and bullocks.

EXPERIMENTAL

The procedure adopted was the same as given in Part I of the present Series [Mukherjee and Kehar, 1949a]. The endogenous urinary nitrogen for sheep was, calculated to be 0.03 gm. per kg. body weight [the figure being 0.0333 gm. according to Harris and Mitchell, 1941] and metabolic faecal nitrogen was computed by the graphical method [Mukherjee and Kehar, 1949c]. The ration consisted of paddy straw or oat straw together with a concentrate mixture to provide a protein level of nine per cent in the case of paddy straw and eight per cent for oat straw. The composition of the concentrate mixture in paddy straw experiment was the same as used in the case of our wheat straw experiments. But in observations on oat straw, the concentrate mixture containing groundnut cake was brought down to the level of colza cake protein by adding starch. The concentrate mixture containing groundnut cake which was used with oat straw consisted of groundnut cake (82 parts) starch (15 parts), calcium carbonate (1.5 parts) and sodium chloride (1.5 parts). The colza cake and mustard cake mixtures contained 97 parts of either of these cakes together with calcium carbonate and sodium chloride (1.5 parts each).

RESULTS AND DISCUSSIONS

(a) Experiment 1. Observations on paddy straw fed with colza or mustard cake

The intake of total nitrogen and net energy as shown in Table I, was slightly higher in the colza cake group as compared with mustard cake group.

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TABLE I

Biological value and digestibility coefficient of the proteins of two oilcakes plus paddy straw in cattle at 9 per cent protein level.

(Metabolism data on daily basis)

Bullock number	Average body weight= W (lb.)	Food dry matter intake (gm.)	Food N		Faecal dry-matter =F.D. (gm.)	F.D. 0.73 W	Nutrient intake* per 1000 lb. body weight		
			Rough-age (gm.)	Concen-trate (gm.)			Starch equi-valent† (lb.)	Digestible pro-tein (lb.)	
Colza cake ration									
L 1	804	5706	22.4	55.6	2356	17.8	5.1	0.74	
Ha 8	1072	8397	32.8	84.7	3258	20.0	6.1	0.96	
Ha 11	980	7242	28.3	75.0	3226	21.2	5.7	0.89	
Mustard cake ration									
L 5	784	4893	10.5	49.3	2238	17.2	4.5	0.64	
Ha 5	1084	8170	32.2	79.9	3251	19.8	5.9	0.90	
Ha 13	956	6753	26.1	68.1	2486	16.6	5.4	0.89	
Bullock number	Food N (total) (gm.)	Faecal N		Urinary N		Nbal-ance (gm.)	Biologi-cal value per cent	Digestibility coefficient	
		Total (gm.)	Metabo-lic (gm.)	Total (gm.)	Endo-genous (gm.)			True	Apparent
Colza cake ration									
L 1	78.0	32.3	18.7	32.4	7.3	+13.3	61.0	82.6	58.6
Ha 8	117.8	43.0	24.4	46.5	9.7	+27.1	62.4	83.4	62.6
Ha 11	103.3	40.2	23.9	41.2	8.9	+21.9	62.9	84.2	61.1
Average	99.6						62.1	83.4	60.8
Mustard cake ration									
L 5	68.8	29.8	17.9	24.9	7.1	+14.1	68.7	82.7	55.9
Ha 5	112.1	43.1	24.3	40.6	9.8	+28.4	67.0	83.2	61.5
Ha 13	94.2	31.5	19.2	35.7	8.7	+27.0	67.0	87.0	66.6
Average	91.6						67.6	84.3	61.3

*Calculated according to the 0.73 power of the body weight.

† Assuming the starch value of the dry matter of the straw and cakes to be 25 per cent and 77 per cent respectively.

In spite of higher energy intake on the colza cake ration, the biological value of its protein (average 62.1 per cent) was lower than that of the mustard cake ration (average 67.6 per cent). The difference in biological value was statistically significant. Mukherjee and Kehar [1949a] pointed out that when colza and mustard cakes were fed with wheat straw as roughage the biological values were identical. The explanation of this unexpectedly high value for the mustard cake ration probably lies in the existence of a marked supplementary relationship between the proteins of paddy straw and mustard cake. The digestibility coefficients of the protein of the two rations were, however, similar.

TABLE II

Biological value and digestibility coefficient of the proteins of two oil cakes plus oat straw in cattle at 8 per cent protein level*

(Metabolism data on daily basis)

Bullock number	Average body wt. = W (lb.)	Food dry matter intake (gm.)	Food N		Faecal dry matter = F.D. (gm.)	F. D. 0.73 W	Nutrient intake† per 1000 lb. body weight	
			Roughage (gm.)	Concentrate (gm.)			Starch equivalent‡ (lb.)	Digestible protein (lb.)
Groundnut cake ration								
K 61	240	1932	9.4	17.2	807	14.7	4.3	0.61
K 240	350	2438	11.8	22.6	1127	15.4	4.1	0.58
K 408	350	2157	10.2	22.6	703	9.6	3.8	0.68
Colza cake ration								
K 334	230	1927	7.5	15.3	930	17.5	4.4	0.44
K 39	398	2428	9.2	23.3	972	12.0	3.8	0.40
K 193	316	2701	10.7	19.3	1090	16.0	4.7	0.47
K 61	246	1962	7.6	16.3	782	13.7	4.2	0.49

*Starch was added in the groundnut cake ration for equalizing the protein content of the concentrates.

†Calculated according to 0.73 power of the body weight.

‡Assuming the starch value of straw, cake and starch to be 36 per cent, 77 per cent and 77 per cent respectively on dry matter basis.

TABLE II—*contd.*

Bullock number	N intake per 1000 lb. body weight† (gm.)	Faecal N		Urinary N		N Balance per 1000 lb. body weight* (gm.)	Biological value	Digestibility coefficient	
		Total (gm.)	Metabolic (gm.)	Total (gm.)	Endogenous (gm.)			True	Apparent
<i>Groundnut cake ration</i>									
K 61	75.0	11.0	6.6	8.6	2.2	+19.7	71.2	83.5	58.6
K 240	73.0	14.4	9.1	15.0	3.2	+10.6	59.4	84.6	58.1
K 408	69.7	9.7	6.9	15.6	3.2	+15.9	58.7	91.5	70.4
<i>Average</i>	72.6					+15.4	63.1	86.5	62.4
<i>Colza cake ration</i>									
K 334	66.7	12.0	7.4	8.5	2.1	+6.7	64.8	79.8	47.4
K 39	62.2	14.0	8.9	7.1	3.6	+21.8	87.2	84.3	56.9
K 103	68.4	15.0	8.8	10.4	2.9	+10.5	68.5	79.3	50.0
K 61	65.0	10.7	6.8	7.2	2.2	+16.3	75.0	83.7	55.2
<i>Average</i>	65.6					+13.8	73.9	81.8	52.4

*Calculated according to 0.73 power of the body weight.

(b) *Experiment 2. Observations on oat straw fed with groundnut cake or colza cake to cattle*

Table II shows the nitrogen metabolism data of Kumaun bullocks fed on oat straw together with a concentrate mixture containing either colza cake or groundnut cake. The energy intake was similar on both rations but the protein level was somewhat lower on the colza cake ration. The average biological value was higher in the latter cake (average 73.9 per cent for colza cake ration as compared with 63.1 per cent for groundnut cake ration) but this difference, although large, was not statistically significant (calculated value of $t=1.60$, tabular value of t at 5 per cent level of significance=2.57); the variation in the individual values was wide in both cases. The difference in the true digestibility was also not statistically significant.

(c) *Experiments 2 (b). Observations on oat straw fed with groundnut or colza cake to sheep*

In this experiment the individual variation in the biological value on the same ration (*vide* Table III) was very high. The average biological values were 48.4

TABLE III

Biological value and digestibility coefficient of the proteins of two oilcakes plus oat straw in sheep at 8 per cent protein level*

(Metabolism data on daily basis)

Sheep number	Average body weight = W. (lb.)	Food dry matter intake (gm.)	Food N		Faecal dry matter = F.D. (gm.)	F.D. 0.73 W (gm.)	Nutrient intake† per 1000 lb. body weight	
			Roughage (gm.)	Concentrate (gm.)			Starch equivalent‡ (lb.)	Digestible protein (lb.)

Groundnut cake ration

1	39	541	2.63	4.83	229	15.3	4.4	0.63
2	37	384	1.76	4.56	207	13.8	3.3	0.51
3	40	537	2.08	4.63	227	15.1	4.4	0.51
4	40	506	1.96	4.28	196	13.1	4.1	3.49

Colza cake ration

5	36	493	1.90	4.32	197	14.4	4.4	0.54
6	32	374	1.35	4.30	145	11.7	4.0	0.59
7	28	348	1.30	3.55	158	13.9	3.9	0.49
8	55	655	2.56	5.33	255	13.5	4.2	0.50

Sheep number	N intake per 1000 lb. body weight† (gm.)	Faecal N		Urinary N		N		Digestibility coefficient	
		Total (gm.)	Metabolic (gm.)	Total (gm.)	Endogenous (gm.)	Balance per 1000 lb. body weight† (gm.)	Biological value	True	Apparent

Groundnut cake ration

1	77.1	3.00	1.87	4.47	0.54	-0.1	37.9	84.9	50.8
2	65.3	2.77	1.80	2.99	0.51	+5.8	53.6	84.7	56.2
3	68.2	3.06	1.87	3.65	0.54	-1.0	42.6	82.0	53.7
4	64.5	2.80	1.72	2.64	0.54	+8.3	59.3	82.7	55.1
Average	68.8					+3.2	48.4	83.6	56.2

Colza cake ration

5	70.4	2.73	1.64	3.00	0.49	+5.6	51.1	82.5	56.1
6	70.6	2.20	1.30	1.87	0.43	+19.8	69.7	84.1	61.0
7	65.9	2.23	1.37	2.04	0.38	+7.9	58.4	82.3	54.0
8	64.7	3.42	2.17	1.97	0.75	+20.5	81.6	84.2	56.7
Average	67.9					+13.4	65.2	83.3	56.9

Foot notes.—Same as in Table II

per cent for oat straw plus groundnut cake and 65.2 per cent for oat straw plus colza cake, the difference being barely significant (calculated value of $t=2.03$, tabular t at 5 per cent level = 2.45). The net energy intake and protein level were, however, identical in both cases. The individual variation in the true digestibility coefficients was negligible and the figures were similar on the two rations.

(d) *Comparison of the biological value and digestibility results on cattle and sheep*

From the data given in Tables II and III it appears that the average figures of the biological value of both groundnut cake and colza cake rations are higher in cattle. Statistically, however, the difference is barely significant on the former ration (average biological value on cattle = 63.1 per cent, average biological value on sheep = 48.4 per cent; calculated value of $t=2.19$, tabular t at 5 per cent level = 2.57) and insignificant in the latter case (average biological value on cattle = 73.9 per cent, average biological value on sheep = 65.2 per cent; calculated $t=1.05$, tabular t at 5 per cent level = 2.45). These results seem to suggest that there is a slight tendency for the absorbed nitrogen to be utilized more efficiently by cattle than by sheep for maintenance purposes. It may, however, be mentioned here that no experimental data are available in the literature on the biological value of the same ration for cattle and sheep, although it is generally assumed that all ruminants behave alike in utilizing the digested nitrogen.

The true digestibility figures given in Tables II and III indicate that the two species of animals digest dietary protein to about the same extent. No published data appear to be available regarding the true digestibility coefficient of feed proteins determined by parallel experiments on cattle and sheep. Some articles giving such figures for apparent digestibility have, however, been recorded. Some of these observations indicate that the two species do not differ in their digestive capacity [Axelsson, 1942; Sears *et al.*, 1942], while other evidences [Forbes *et al.*, 1937; Wolberg *et al.*, 1940] show that sheep digest feed protein more efficiently than cattle.

(e) *Comparative study of the biological value and true digestibility of the proteins of the straws of wheat, paddy and oats*

In Table IV relevant observations have been rearranged so as to facilitate comparison. The data included here have been taken from Tables I and II and also from the first paper of this Series [Mukherjee and Kehar 1949a]. The true digestibility figures have not been included in the Table, since the figures vary within a narrow limit, *viz.* 82 per cent to 88 per cent) and no significant difference exists between them. Table IV shows that oat straw is appreciably superior to wheat straw, when fed either with groundnut cake or colza cake. Paddy straw also appears to be significantly better than wheat straw when consumed in combination with colza cake; but this effect may be partly or wholly due to the comparatively high caloric intake in the paddy straw experiment since in all other experiments the energy intake was maintained at a lower level (*vide* Table IV).

The biological values (*viz.* 62 to 74 per cent) of the proteins of the oat straw and paddy straw rations referred to in Table IV compare favourably with those (65 per cent and 71 per cent) for adult cattle [Morris, 1935] of similar rations in which oat straw was used in combination with maize germ meal and rye flour respectively.

Working on growing lambs, Turk *et al.* [1934] found the biological value of red clover hay and alfalfa hay (with corn or starch) to be about 80 per cent at 10 per cent protein level; but other workers [Miller and Morrison, 1939; Miller *et al.*, 1937; Johnson *et al.*, 1942] give a lower value, viz. 56 to 64 per cent for alfalfa hay, timothy hay and red top hay (in combination with concentrates such as corn, soyabean meal and linseed meal) at 10-12 per cent protein level. No information appears to be available in literature regarding the biological value of the proteins of rations having wheat straw and paddy straw as the roughage component. Snell *et al.* [1945], however, report that in a fattening ration for steers, paddy straw is more economical than hay alone; this probably indicates that paddy straw protein is of good quality.

TABLE IV

Comparative study of the biological value of wheat, oat and paddy straws

Ration				Biological value per cent		Statistical significance of difference between pairs
Roughage	Oilcake	Protein level per cent	Starch equivalent intake lb.* per 1000 lb. body weight.	Mean	S.E.†	
A. Wheat straw	Groundnut	8.5	4.1	41.5	0.7	A, B = Highly significant
B. Oat straw	"	9.0	4.1	63.1	4.1	
C. Wheat straw	Colza	9.3	4.2	48.3	2.2	C, D = Highly significant
D. Paddy straw	"	8.7	5.6	62.1	0.6	
E. Oat straw	"	7.6	4.3	73.9	4.9	C, E = Highly significant

*Calculated on the basis of the 0.73 power of the body weight

†Standard error

SUMMARY

The average biological values of the proteins of paddy straw in combination with colza cake and mustard cake as determined on cattle were 62 per cent and 68 per cent. Parallel experiments conducted on cattle and sheep gave the average values for oat straw plus groundnut cake as 63 per cent and 48 per cent respectively; the corresponding values of the colza cake combination were 74 per cent and 65 per cent respectively. The true digestibility of the proteins of these rations varied within a narrow limit, viz. 82 per cent to 86 per cent. Statistical analysis of the data shows that the protein of oat straw rations was superior to that of the corresponding wheat straw diets. Paddy straw protein, too, is considered to be of good quality.

The results further suggest that there is a slight tendency for the absorbed nitrogen to be utilized more efficiently by cattle than by sheep for maintenance purposes.

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FEEDING VALUE OF SUGARCANE TOPS

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IT has been estimated* that in India an area of about 4,234 thousand acres was under sugarcane during the year 1943-44. If the average yield is taken as 12.96 tons per acre [Dutt, 1946], the total yield of sugarcane is expected to be 54,880 thousand tons per year. The amount of sugarcane tops will be 6,098 thousand tons on the basis that the tops weigh one-ninth of the cane. With common cultivated grasses, it would require about 833 thousand acres to produce the same amount of green fodder, if the yield per acre is taken as 7.32 tons (200 maunds), the average figure for *jowar* fodder (*Sorghum vulgare*). According to Wright [1937], the total acreage under fodder crops in India including areas now under Pakistan, during 1935 was 10,200 thousand acres. Thus, it will be seen that the amount of green forage available as sugarcane tops is equivalent to the produce of about one-twelfth of the area under fodder crops.

For the United Provinces where the acreage under sugarcane is nearly 50 per cent of the total area in India, the question of utilization of sugarcane tops is of great importance. It is expected that 2,240 thousand acres*, the area under sugarcane in this province, can produce 3,225 thousand tons of tops. It will require 440.5 thousand acres under *jowar* to produce this amount of green fodder, whereas, the total area under fodder crops in this province is only 1,604 thousand acres, 3.6 times more than the area equivalent to the amount of sugarcane tops.

Though there is scarcity of green fodder in the country, sugarcane tops are not properly utilized as cattle feed and instead, a major portion is burnt as fuel during *gur* (raw sugar) manufacture. Ignorance of its feeding value may be the main cause of such wastage. Practically no information is available about the nutritive value of sugarcane tops. The Department of Agriculture-United States, in Circular No. 284 recommended tops and leaves of common sugarcane as a satisfactory forage for livestock. Morrison [1936] has recorded the analysis of one sample of sugarcane tops but the digestion coefficient of the nutrients were not available. Therefore, experiments were conducted with the object in view that the knowledge of the feeding value of sugarcane tops might help the growers to make its economic use.

EXPERIMENTAL

To study the value of sugarcane tops as forage, tests were carried out with cane Co. 312 at Madhurikund Cattle Farm, Mathura.

Composition

The collection of weekly sample of sugarcane tops was started from 1 February, 1946 when the crop was ripe and it continued till the third week of April. The dried samples were sent to Bharari, Jhansi, where the representative samples for

* Estimate of area and yield of principal crops in India, 1941-42, 1942-43 and 1943-44, Department of Commercial Intelligence and Statistics, India.

the periods 1 February to 7 March, 8 March to 11 April and 14 April to 22 April were analyzed. The figures obtained have been shown in Table I.

TABLE I

Per cent composition

	Dry matter	On dry basis				
		Organic matter	Protein	Ether extract	Fibre	N. F. E.
Sugarcane tops (Madhurikund, Mathura)						
1 February to 7 March	33.70	88.55	4.64	1.96	34.72	47.23
8 March to 11 April	28.40	87.84	4.83	1.95	34.25	46.81
14 April to 22 April	39.87	87.54	4.50	3.05	31.87	48.12
Morrison's figure	28.50	92.60	5.30	1.40	31.20	54.70
<i>Jowar fodder</i>						
Bharari, Jhansi, average, prime to mature September to November	35.10	88.91	3.90	1.12	29.22	54.07
Etawah average, prime to mature September to November	33.80	90.88	3.15	1.50	30.16	56.07
Madhurikund (mature) November	46.20	90.20	5.18	1.16	30.74	53.12
<i>Napier grass</i>						
Bharari, Jhansi, average, September to November	28.40	86.13	5.85	1.13	30.91	42.24
Etawah, average, September to November	30.90	89.24	2.34	1.03	35.63	50.24
Madhurikund (Mathura) November	34.10	87.08	2.78	1.36	32.87	50.07

It is observed that the composition of sugarcane tops did not vary to a great extent from February to April, *i.e.*, ripe to over-ripe stage. The variation in the dry matter content during March, might have been influenced by the irrigation of the plot. The figures recorded by Morrison *et al.* have also been shown in Table I for comparison. It appears that the sugar content of this sample was higher, which resulted in the slightly higher nitrogen-free extract content. The data obtained by this section for *jowar* fodder and Napier grass, two cultivated fodders, have also

been included. The composition shows that there may not be much difference in the nutritive value of the three forages.

DIGESTIBLE NUTRIENTS

To find out the digestible nutrients of sugarcane tops, digestion test was carried out with three Haryana cows for a period of nine days towards the end of a feeding test. The cows were fed sugarcane tops *ad lib.* and linseed cake to supplement protein. The average daily consumption of different feeds by an animal is shown in Table II. The composition of the feeds and faeces during the digestion test has been recorded in Table III. The digestion coefficients obtained for the mixed ration of sugarcane tops and linseed cake and also the calculated coefficients arrived at for sugarcane tops have been shown in Table IV.

TABLE II

Average daily intake of feeds and excretion of faeces on dry basis

Cow number	132		969		74	
Feeds	Average consumption per day	Average faeces per day	Average consumption per day	Average faeces per day	Average consumption per day	Average faeces per day
Sugarcane tops	9640.0	..	9597.0	..	10231.0	..
Linseed cake	722.8	..	722.8	..	722.8	..
<i>Total</i>	10362.8	3438	10319.8	3184	10953.8	3313

TABLE III

Composition of feeds and faeces on dry basis

—	Organic matter	Protein	Fat	Fibre	N. F. E.
Sugarcane tops	87.5	4.5	3.1	31.0	48.0
Linseed cake	90.6	35.6	2.4	8.4	44.3
Faeces of 132	81.6	6.4	2.0	25.0	47.3
„ 969	81.2	7.4	2.2	25.2	46.4
„ 74	80.7	7.0	2.3	25.8	45.8

TABLE IV
Digestion coefficient of nutrients

Particulars	Organic matter	Protein	Ether extract	Fibre	N. F. E.
<i>Mixed ration of sugarcane tops and linseed cake</i>					
Cow 132	69.6	68.7	77.3	71.9	73.3
969	71.9	66.5	76.7	74.6	70.8
74	72.5	68.2	77.0	74.5	71.9
<i>Average</i>	71.3	67.8	77.0	73.7	72.0
<i>Sugarcane tops alone</i>					
Cow 132	81	58	76	72	73
969	72	55	76	75	70
74	72	58	76	75	71
<i>Average</i>	75	57	76	74	72
Elephant grass	..	62	59	63	65
Guinea grass	59	58	43	61	52
<i>Jowar</i>	58	44	44	59	60
Maize	..	61	65	70	76

The approximate liveweights were 750, 750 and 850 lb. of cows 132, 969 and 74 respectively and hence the daily dry matter intake was 1.38, 1.38 and 1.37 kg. per 100 lb. liveweight, which was quite satisfactory for cows with poor milk yield. This shows that the sugarcane tops, which were the main source of dry matter, were relished by the cows.

There was not much variation in the digestion coefficients obtained with the three animals. In Table IV the digestibility of the nutrients of sugarcane tops has also been compared with the data recorded by Sen [1938] for some cultivated fodders. It is observed that the nutrients of sugarcane tops are much better digested than the common forage *jowar* and are also superior to Elephant grass and Guinea grass. For better comparison of their feeding value, the total digestible nutrients of these forages have been tabulated in Table V.

The tops used for the digestion test contained, on an average, 39.87 per cent dry matter. When calculated on dry basis, the figures obtained for digestible nutrients were 3.21 for protein and 66.76 for total digestible nutrients. The digestion test with the sugarcane tops was conducted during the month of April when the canes were over-ripe. It is observed that tops of over-ripe Co. 312 cane are nearly equal to maize in their nutritive value and compare favourably with Elephant grass, Guinea grass and *jowar*.

TABLE V

Digestible nutrients per 100 lb. dry material (lb.)

	Crude protein	Ether extract	Carbohydrates	Total digestible nutrients
Sugarcane tops	3.21	2.35	58.26	66.76
Elephant grass	3.85	1.33	48.54	55.39
Guinea grass	4.05	0.70	45.96	51.59
Jowar (prime)	2.55	0.70	50.79	54.91
Maize	4.44	1.16	61.01	68.02

Feeding test with sugarcane tops

From February onward stock owners usually feed straws along with some oil-cake or concentrate to their animals. Hence, to study the effect of sugarcane tops on milk yield and also to determine the saving in concentrate in the usual straw and cake ration when sugarcane tops were fed, test was conducted at Madhurikund Cattle Farm with six Haryana cows. The animals were fed *dub* grass (*Cyanodon dactylon*) hay and linseed cake during the pre-experimental period. They were divided into comparable groups of three each after the preliminary feeding. The treatments, (i) linseed cake to supplement the required protein along with wheat straw and (ii) sugarcane tops and half the amount of linseed cake necessary to provide protein if the animals had been on wheat straw, were tried in a switch over design. The group receiving wheat straw in the first cycle was fed sugarcane tops in the second and *vice versa*. Each period lasted for 35 days. Both straws and tops were fed *ad lib*. Usually no digestible protein is available from wheat straw, whereas, it was expected that sugarcane tops will provide about 3 per cent digestible protein on dry basis and that the amount of tops consumed might provide nearly half the protein required for the animals. The animals when on wheat straw, were provided with 500 gm. sugarcane tops for supplementing vitamin. All the animals were given 20 gm. salt per head per day.

MILK PRODUCTION AS INFLUENCED BY THE TREATMENTS

The production of milk during the pre-experimental period and also during both the cycles of the experiment has been recorded in Table VI.

TABLE VI

Milk production as influenced by sugarcane tops (lb.)

Animal number	953	316	79	Total per day	262	132	969	Total per day
<i>Preliminary period</i>								
Weeks	Cake with grass hay				Cake with grass hay			
1	8.1	7.7	6.0	21.8	7.7	7.6	8.7	24.0
2	7.5	6.4	5.8	19.7	6.8	7.5	7.7	21.7
3	7.4	5.9	5.2	18.5	6.0	7.5	7.4	20.9
4	7.2	4.6	5.2	17.0	6.0	7.7	7.4	21.1
<i>Experimental period</i>								
	First cycle Reduced cake with sugarcane tops				Full cake with wheat straw			
1	7.6	5.7	6.4	19.7	2.8	7.7	7.3	17.8
2	7.6	6.2	6.7	20.5	2.6	8.2	7.1	17.9
3	7.4	6.1	6.8	20.3	3.1	7.8	7.1	18.0
4	7.3	5.5	6.2	19.0	3.0	7.4	6.5	16.9
5	7.4	6.0	6.7	20.1	3.2	7.2	7.1	17.5
	Second cycle Full cake with wheat straw				Reduced cake with sugarcane tops			
6	7.0	5.7	6.9	19.6	2.6	6.9	7.2	16.7
7	6.6	6.1	6.6	19.3	3.3	6.8	6.7	16.8
8	6.2	5.1	5.9	17.2	5.0	7.2	6.8	19.0
9	6.1	5.3	5.6	17.0	5.6	7.6	7.0	20.2
10	6.4	4.7	4.1	15.2	5.3	7.0	6.7	19.0

The grass hay fed was not of inferior quality but Table VI above shows that there was a definite increase in the milk yield when the ration was changed from grass hay to sugarcane top with reduced oilcake and a marked decrease when the change was to the full amount of cake to supplement protein with wheat straw. But the effect of the change from one experimental ration to the other and *vice versa* was not in the same order with all the animals.

If the first week of the each cycle is not taken into account, to avoid the residual effect, it is found that all the six animals produced, during the last four weeks of the treatment, 1,084 lb. milk with sugarcane tops and reduced cake and 973 lb. with full cake and wheat straw. The milk yield figures indicate that *dub* grass hay and

full linseed cake is inferior to sugarcane tops and half linseed cake, but superior to wheat straw and full linseed cake.

The data were analyzed statistically and it was found that the average milk yield per head was 6.5 lb. with sugarcane tops as compared to 5.8 lb. with the other treatment. But, the difference was not significant when compared to the variations due to individuality.

Fat content of milk

Fat was estimated for three consecutive days, both morning and evening, towards the end of each cycle. The data have been summarized in Table VII.

TABLE VII

Fat percentage of milk

Brand number	953	316	79	969	132	262
Average of preliminary period	Grass hay and cake 5.2 4.2 4.3			Grass hay and cake 4.8 4.3 4.6		
Average of experimental period	Reduced cake and sugarcane tops 6.0 5.8 4.7			Full cake and wheat straw 5.1 5.0 5.0		
	Full wheat 6.7 Cake and straw 6.0 5.6			Reduced cake and sugarcane tops 5.7 5.4 5.0		

The average percentage of fat in milk was 5.4 with sugarcane tops and 5.6 with wheat straw. The difference was not significant, more so, when the yield of milk is taken into accounts as percentage of fat increases with the decrease in milk yield independent of the effect of food.

Milk yield as influenced by the digestible nutrients

The consumption of food on dry basis was much higher when the animals were having sugarcane tops, which may be attributed to its palatability. The average consumption of feeds per head per day was as follows:

—	Wheat straw	Sugarcane tops	Linseed cake	Total dry matter
	lb.	lb.	lb.	lb.
Reduced cake and sugarcane top treatment	..	16.9	1.4	18.3
Full cake and wheat straw treatment	10.8	0.3	2.9	14.0

Consumption of about 4.3 lb. more dry matter per head per day cannot but have some effect on the production. Data obtained from the digestion test also show the superiority of the sugarcane top treatment in total digestible nutrients, though, the amount of oilcake fed was nearly half of that present in the other treatment. The digestion test was conducted with three cows under each treatment. The average coefficients obtained are recorded in Table VIII.

TABLE VIII
Per cent digestibility of nutrients

	Protein	Ether extract	Fibre	N. F. E.
Sugarcane top total ration	67.8	77.0	73.7	72.0
Wheat straw total ration	75.1	74.5	57.8	59.2
Sugarcane top	56.9	76.0	73.9	71.6

The digestibility of fibre and N. F. E. of the total ration was higher and that of protein lower in the sugarcane top treatment. This was due to the higher digestibility of fibre and N. F. E. of the sugarcane tops as compared to straw and lower digestibility of the protein as compared to that of linseed cake, which was the main source of digestible protein in the straw treatment. The digestibility of the sugarcane tops was calculated by deducting the amount digested from the cake. Both the intake and digestibility were superior with the sugarcane top treatment and naturally the consumption of total digestible nutrients was also higher. The average daily intake of total digestible nutrients by the two treatments was as shown in Table IX.

TABLE IX
Average daily intake of total digestible nutrients

	Intake from roughage	Intake from cake	Intake from tops as vitamin supplement	Total intake
Reduced cake and sugarcane top treatment	11.40	0.92	..	12.32
Full cake and wheat straw treatment	5.76	1.82	0.22	7.80

The average daily intake of T. D. N. in the sugarcane top treatment was 12.32 as compared to 7.80 with the full cake ration. This shows that even with a reduction of 50 per cent oilcake, feeding of sugarcane tops provides more nutrients than cereal straw supplemented with full amount of cake. The total milk produced was more with the sugarcane tops. Though, in the present test the difference was not

statistically significant, it can be assumed that better production will be achieved with tops when the quantity of concentrate necessary with straw is even halved.

ECONOMY OF FEEDING SUGARCANE TOP

The sugarcane was utilized by the farm for the preparation of raw sugar and the tops were fed to the animals. Wheat straw had to be purchased from the neighbouring villages at the rate of Rs. 3-12 per maund. As one of the roughages was home grown and the other purchased it is not possible to compare their cost. But, if the amount of the cake which is usually purchased by the stock owners is considered, it is observed that for the production of 1084 lb. milk when the animals were under sugarcane top treatment, the consumption of linseed cake was 253.6 lb. as against 539.5 lb. for production of 973 lb. of milk with the wheat straw ration. This amounts to that 23.4 lb. linseed cake supplement is required for 100 lb. milk yield with sugarcane tops as roughage as compared to 53.5 lb. with wheat straw.

SUMMARY

Experiments to find out the digestible nutrients and the feeding value of the tops of sugarcane Co. 312 for milk yield were conducted. Haryana milch cows were used for these tests. The cane was over ripe during the digestion test.

Sugarcane tops were fed along with some linseed cake. The digestible coefficients were calculated after deducting the digestible nutrients provided through oilcake. The average coefficients arrived at from three cows were 57, 76, 74 and 72 for protein, ether extract, fibre and N-free-extract respectively. The tops used for the digestion test contained, on an average, 39.9 per cent dry matter. When calculated on dry basis, the figures for digestible nutrients obtained were 3.2, 2.4, 58.3 and 66.8 per cent for protein, ether extract, carbohydrates and total digestible nutrients respectively.

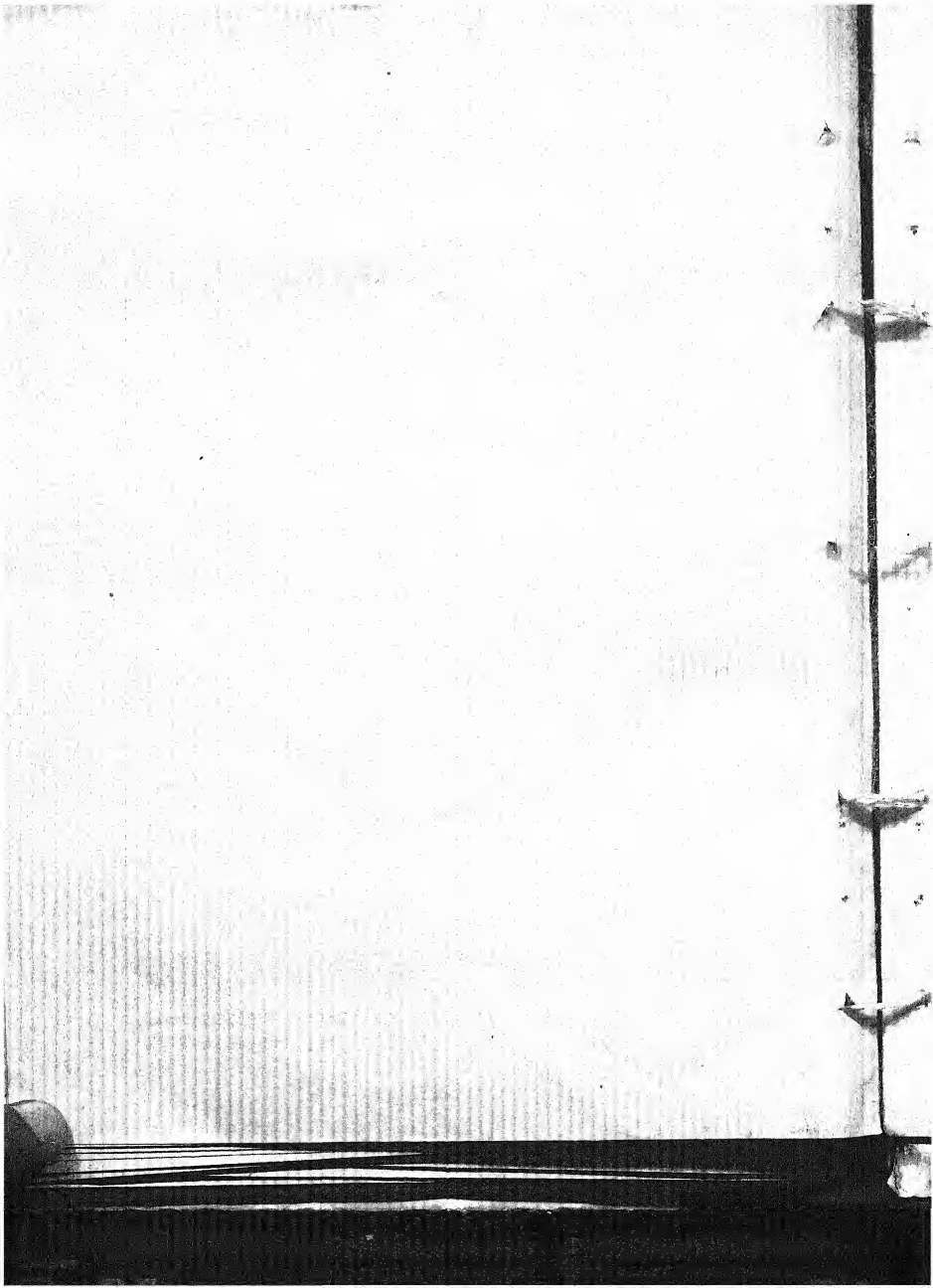
The effect of feeding sugarcane tops on milk yield was studied with six animals for a period of 35 days. The results show that the milk yield is quite satisfactory even when the amount of oilcake supplement necessary with wheat straw is reduced, by 50 per cent while feeding the sugarcane tops.

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DETECTION OF ADULTERATION OF GHEE (BUTTER-FAT)

LINOLEIC ACID CONTENT OF GHEE AND FATTY ACID COMPOSITION OF BUFFALO GHEE

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A STUDY [Achaya and Banerjee, 1945] of the fatty acid constitution of typical Indian buffalo *ghees* revealed that the only figure which had a definite value of detecting adulteration was the content of linoleic acid. This varied in genuine *ghee* to a small degree, but the percentage of the acid in all the *ghees* was of the order of 1.5 per cent. The percentage of this acid in some oils, which are used both for feeding animals and for direct adulteration of *ghee*, is as follows: Groundnut oil, 20.0; cottonseed oil, 48.0; coconut oil, 1.5; and hydrogenated groundnut oil (I. V., 50) consistency akin to that of *ghee* 10.0. Genuine *ghee* is very poor in linoleic acid compared to these oils and hydrogenated products; even 10 per cent adulteration would cause the content of this acid to shoot-up from 1.5 per cent to a very high figure except for coconut oil which fortunately can be detected even in fairly small amounts by the Reichert Polenske figures. It is necessary to study thoroughly the linoleic acid contents of various types of *ghees* which cover the limits of natural variations due to breed, feed, season, etc. Unless it is proved and confirmed from extensive number of samples that linoleic acid content in genuine *ghee* is never more than two per cent it cannot be recommended as a legal standard.

The effects of feed on *ghee* are well known. It is known that due to oilseed or cake feeding, *ghee* constants are altered. Taking advantage of this, adulterated *ghee* whose constants are abnormal is passed off as resulting from such feeding. Thus, a person mixes *ghee* with hydrogenated cotton seed oil or groundnut oil and claims that the alteration or abnormality is due to cotton seed or groundnut cake feeding. Cotton seed is the only oil seed that is fed whole and others are fed as oilcakes. Again cotton seed feeding produces sub-normal constants of *ghee*. A detailed examination of buffalo *ghee* and body fat has shown that fortunately the linoleic acid of cotton seed and other oils is not absorbed to any extent in the butter fat. If iodine and thiocyanogen values of *ghee* are determined, the linoleic acid of *ghee* can be determined. Now, if hydrogenated cotton seed groundnut oil is added, the linoleic acid is bound to be more than two per cent which is not the case with natural *ghee*.

There are three exclusive cotton seed areas, the Central Provinces, Gujerat and Kathiawar. Genuine samples were collected from these areas and from the cotton-seed areas of the Punjab, Sind, U. P., C. P. and Bombay. More than 60 per cent of the samples were collected in the dry and hot seasons and the remainder in other parts of the year. About 300 samples from such areas and 100 from other areas were collected.

Of the vegetable oils, linseed oil has a very high iodine value, that is a high percentage of linoleic and linolenic acids. Animals were fed with linseed meal to find out if such special feeds gave rise to increase in the iodine and thiocyanogen values. With the kind help of the Indian Dairy Research Institute, Bangalore, cows and buffaloes were fed linseed meal in their ration and the butter-fat analyzed. The iodine and thiocyanogen values of linseed meal fed ghees are given below :

TABLE I
Iodine and thiocyanogen values of linseed-meal-fed ghees

		R.M.	P.V.	I.V.	Thio- cyanogen value	Linoleic acid per cent
Cow ghee	Control stock diet	23.3	1.8	32.5	33.4	1.08
	Experimental II week	23.9	1.4	36.0	37.2	1.30
	Experimental III week	22.9	1.3	36.1	37.3	1.30
Buffalo ghee	Control stock diet	30.06	2.0	25.8	26.9	1.2
	Experimental II week	28.0	1.6	28.5	29.6	1.2
	Experimental III week	28.0	1.5	29.0	30.0	1.12

Composition of concentrate in control stock diet

Oats	55 per cent
Groundnut cake	20 per cent
Wheat bran	15 per cent
Gram husk	10 per cent

Composition of concentrate during experimental period

Oats	60 per cent
Linseed	25 per cent
Wheat bran	15 per cent

Kaufmann [1925] first suggested the determination of iodine and thiocyanogen values of fats and oils or fatty acid esters to determine oleic acid from mixtures of oleic, linoleic and linolenic acids. The method has been given a fair trial [Waterman and Bertram; 1929; Jamieson and Baughman, 1930; Griffiths and Hilditch, 1934 and Hilditch and Murthi, 1940], as doubts were expressed by some workers [Van der Veen, 1931; Smith and Chibnall, 1932]. The procedure can be made satisfactory when carried out under controlled conditions [Hilditch and Murthi, 1940]. Moisture should be completely excluded from the reagents and apparatus. Standardized conditions of procedure at all stages of analysis are essential to get accurate and satisfactory data. Estimations of iodine and thiocyanogen values of butter-fat have been found to indicate that the linoleic acid glyceride content is practically constant, while the oleic glyceride is the variable factor.

Iodine value was determined by the pyridine sulphate dibromide method [Rosenmund and Kuhnemann, 1924, 1925]. For the determination of thiocyanogen value the *A. O. C. S.* method was followed.

The following precautions are necessary for the determination of the thiocyanogen value. Lead thiocyanate was prepared by adding lead nitrate (33.1 gm.) dissolved in 70 c.c. of water to 19.4 gm. of pure potassium thiocyanate in 50 c.c. of water. The precipitated lead thiocyanate was filtered off and washed successively with water, alcohol and ether. It was dehydrated for two weeks over phosphorus pentoxide in an evacuated desiccator. Glacial acetic acid (99 per cent) was refluxed with 15 per cent acetic anhydride for three hours, cooled and kept for at least a week before use. All the apparatus and filter paper used in the preparation of the reagent were dried immediately before use at 110°C. for an hour.

Dry bromine 8.4 gm. was weighed into a 250 ml. graduated flask and dissolved in 100 c.c. of pure dry carbon tetrachloride. The flask was then filled to the mark with anhydrous acetic acid. In a glass stoppered bottle 25 gm. of pure dry lead thiocyanate were weighed and kept suspended in the prepared acetic acid for at least a week before use. The bromine solution was added to the lead thiocyanate in small quantities at a time with vigorous shaking, taking care that decolorization was complete before each addition of the solution. After thorough shaking the solution was filtered into a dry brown glass stoppered bottle and used for the determination of the thiocyanogen value.

Ghee 0.1—0.2 gm. was put into a 250 ml. glass stoppered flask and 25 ml. of the thiocyanogen solution added from a pipette and allowed to stand in the dark for 24 hours 10 ml. of 20 per cent potassium iodide solution was then added quickly and shaken immediately to prevent hydrolysis of the thiocyanogen solution. Water 100 ml. was then added and the liberated iodine titrated with standardized 0.1 N sodium thiosulphate, using starch as indicator. At least two blank determinations were made alongside the determination on sample.

The iodine value of oleic acid is 89.9 and that of linoleic acid, 181.14 while the thiocyanogen values are 89.7 and 95.7 respectively. If x is the percentage of oleic acid and y , the percentage of linoleic acid then,

$$x \times 89.9 + y \times 181.14 = I.V.$$

$$x \times 89.7 + y \times 95.7 = T.V.$$

$$\therefore 85.44y = I.V. - T.V.$$

$$y = \frac{100}{85.44} \times (I.V. - T.V.)$$

$$\text{Hence, percentage of linoleic acid} = \frac{100}{85.44} \times (I.V. - T.V.)$$

The iodine and thiocyanogen values of the samples analyzed are given in Table II.

TABLE II
Analytical constants of ghee

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
1	Wardha	April	17.6	0.6	35.5	34.6	1.08
2			18.2	0.8	34.4	32.7	2.0
3			18.2	0.8	34.4	33.4	1.2
4			16.4	0.6	35.2	34.1	1.32
5			18.0	0.9	35.5	33.8	2.0
6			16.7	1.0	28.0	27.1	1.0
7		May	17.8	0.6	33.6	32.4	1.44
8			20.2	1.0	31.9	29.3	2.6
9			20.9	0.7	28.3	27.6	0.84
10			25.6	1.7	36.8	34.9	2.28
11		June	28.6	2.1	36.2	35.1	1.32
12			23.9	0.8	31.0	30.0	1.2
13			28.0	1.9	35.5	34.3	1.44
14			18.5	2.0	38.8	36.9	2.28
15		July	22.9	1.7	41.1	40.1	1.2
16			23.2	1.6	39.8	38.9	1.08
17			24.0	1.6	36.7	35.3	1.08
18		August	20.5	2.3	37.3	36.0	1.56
19			19.9	1.9	37.2	36.1	1.32
20		September	20.6	2.2	36.8	35.1	2.0
21			28.3	2.5	25.9	24.2	2.0
22			27.8	1.9	29.8	28.5	1.56
23		October	28.1	2.0	26.0	24.3	2.0
24			18.5	2.0	38.8	37.6	1.44]
25			18.2	0.7	35.0	34.0	1.2
26		November	17.6	0.4	34.5	33.6	1.08
27			16.9	0.5	35.2	34.0	1.44
28			18.5	2.0	38.8	36.9	2.28
29			19.9	1.8	37.4	35.9	1.8

TABLE II—*contd.*

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
30	Wardha— <i>contd.</i>	December	20.1	1.0	35.0	35.0	1.2
31			20.8	1.3	39.8	38.6	1.44
32			21.8	0.7	42.7	41.8	1.08
33		January	17.8	0.6	33.5	32.4	1.32
34			20.2	1.0	31.9	30.1	2.16
35		February	23.0	1.7	41.0	40.0	1.2
36			22.9	1.7	40.1	39.1	1.2
37		March	27.8	0.7	28.3	27.0	1.56
38			26.0	1.4	35.4	34.3	1.32
39	Junagadh	April	16.4	1.1	41.3	40.0	1.56
40			17.6	0.9	35.1	34.0	1.32
41			16.8	1.0	28.0	26.5	1.80
42		May	19.4	0.9	33.1	32.6	0.60
43			15.0	1.1	37.8	36.3	1.80
44			14.6	1.0	36.8	34.9	2.28
45		June	20.5	2.0	36.4	35.4	1.2
46			21.0	1.0	36.8	34.9	2.28
47			20.8	2.1	36.9	35.0	2.28
48		July	19.4	0.7	34.0	33.0	1.2
49			20.1	1.8	35.0	34.1	1.09
50			20.4	1.6	35.5	34.2	1.56
51		August	20.5	2.2	37.3	35.7	1.92
52			20.4	2.1	37.0	36.0	1.2
53		September	30.1	1.7	36.3	35.8	0.60
54			32.0	1.4	30.9	30.0	1.08
55		October	25.9	1.6	30.8	29.7	1.32
56			24.8	1.7	30.7	29.8	1.08
57		November	20.4	0.6	33.4	32.0	1.68
58			20.2	0.5	32.6	31.4	1.44

TABLE II—*contd.*

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
59	Junagadh— <i>contd.</i>	December	16.8	0.4	37.8	36.0	2.16
60			15.7	0.4	36.9	35.2	2.04
61		January	15.9	0.5	37.0	35.4	1.92
62			17.4	1.0	39.9	38.3	1.92
63		February	17.3	1.3	37.5	36.0	1.80
64			21.6	3.0	39.8	38.7	1.32
65		August	20.7	0.9	44.2	43.0	1.44
66			20.6	2.4	43.0	42.0	1.2
67		Assam	20.8	2.1	42.1	41.0	1.3
68			20.6	2.3	41.8	40.6	1.44
69		September	20.8	2.0	42.0	40.7	1.56
70			17.8	2.1	41.0	40.1	1.08
71		October	17.6	1.9	40.8	39.9	1.08
72			17.8	2.1	40.9	39.9	1.32
73		Bangalore (I.D.R.I.)	16.9	1.8	43.0	41.0	1.32
74			20.3	2.1	28.8	27.9	1.08
75		May	27.3	2.0	30.2	28.4	2.0
76			28.1	1.9	29.0	27.4	1.92
77			28.6	2.3	29.1	27.4	2.0
78			28.4	2.1	28.8	27.4	1.68
79		June	27.6	2.3	30.2	28.4	2.0
80			27.4	2.0	30.1	28.6	1.8
81			27.6	2.0	30.3	29.0	1.56
82			26.9	1.9	30.2	28.5	2.0
83		July	27.5	2.4	29.9	28.3	1.92
84			27.4	2.7	30.2	28.4	2.0
85			30.8	2.0	28.6	26.9	2.0
86			30.7	2.1	29.0	27.9	1.32
87			30.8	2.1	28.8	27.0	2.0

TABLE II—*contd.*

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
88	Bangalore (I. D. R. I.) — <i>contd.</i>	July— <i>contd.</i>	30.4	1.9	29.0	27.7	1.56
89			31.0	1.0	32.0	31.0	1.2
90			33.1	1.2	34.0	32.7	1.56
91			32.6	1.3	35.0	34.0	1.2
92		August	17.5	1.6	33.3	32.1	1.44
93			16.9	1.4	34.0	32.2	2.0
94			17.4	1.3	34.1	32.4	2.0
95			17.0	1.5	33.6	32.3	1.56
96		September	23.0	1.6	33.5	32.3	1.44
97			22.8	1.6	34.0	32.9	1.32
98			23.0	1.4	33.4	31.6	2.0
99			23.2	1.1	34.0	32.2	2.0
100		October	24.0	1.3	35.0	34.0	1.2
101			24.0	1.4	35.2	34.1	1.32
102			22.6	1.4	34.8	33.0	1.08
103			22.4	1.1	34.4	33.0	1.68
104		November	22.4	1.0	34.4	33.0	1.68
105			22.0	1.3	33.6	31.8	2.0
106			21.9	1.4	33.7	31.9	2.0
107			22.0	1.3	33.6	31.9	2.0
108		December	24.8	1.1	34.0	32.3	2.0
109			24.6	1.2	34.2	32.8	1.68
110			24.6	1.1	34.0	32.5	1.8
111			24.4	1.1	33.8	32.0	1.68
112		December	30.4	2.6	31.2	39.3	2.06
113			30.8	2.6	30.8	29.0	2.06
114			30.4	2.2	32.7	31.1	1.92
115			30.6	2.2	31.6	30.6	1.2
116			30.1	2.1	31.1	30.0	1.32

TABLE II—*contd.*

Serial No.	Sample	Month	R. M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
117	Bangalore (I. D. R. I.) — <i>contd.</i>	January	30.4	2.2	32.6	30.8	2.06
118			27.4	1.2	32.6	31.0	1.92
119			27.6	1.0	32.5	31.0	1.9
120			27.4	1.1	32.6	31.1	1.8
121			27.5	1.2	32.6	31.1	1.8
122		February	31.2	1.7	29.9	27.9	2.4
123			31.0	1.7	30.0	28.0	2.4
124			29.1	1.6	28.8	26.9	2.28
125			29.1	1.6	28.9	27.2	2.04
126			29.1	1.8	28.8	26.9	2.28
127		March	29.0	1.6	28.7	27.0	2.04
128			29.0	1.8	28.8	27.0	2.06
129			36.4	1.7	29.4	27.6	2.06
130			36.4	1.6	30.0	28.2	2.06
131			29.2	1.8	28.9	28.0	1.08
132			29.3	1.6	29.0	28.0	1.2
133			30.0	1.6	30.1	29.1	1.2
134			17.8	0.6	33.6	32.1	1.8
135			17.9	0.6	33.6	32.0	1.9
136			20.2	1.0	31.9	30.8	1.3
137	Nagpur . . .		26.8	0.7	28.1	26.9	1.44
138	..	27.9	0.7	28.3	26.9	1.68	
139		26.0	1.3	28.2	26.6	1.9	
140		25.6	1.3	36.8	34.9	2.08	
141		28.9	0.7	32.0	31.0	1.2	
142		30.8	0.7	32.3	31.1	1.44	
143		29.0	2.0	36.0	35.0	1.20	
144		28.6	2.1	36.2	35.0	1.44	
145		28.6	2.1	36.2	34.8	1.68	

TABLE II—*contd.*

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
146	Nagpur— <i>contd.</i>	..	23.0	1.7	41.1	40.1	1.2
147			22.9	1.7	41.1	40.1	1.2
148			20.4	2.3	37.3	36.0	1.56
149			20.3	2.2	37.3	36.0	1.56
150			28.3	2.4	25.9	24.2	2.0
151			28.3	2.4	26.0	25.0	1.2
152			27.9	2.3	26.1	25.1	1.2
153			18.5	2.0	38.8	37.9	1.08
154			18.9	2.0	38.6	37.0	1.92
155	Dokri-Sind	..	28.3	1.1	30.9	29.8	1.32
156			27.3	1.0	29.8	28.8	1.2
157			21.8	0.7	42.7	41.9	0.72
158			20.0	1.0	35.0	34.0	1.2
159			25.0	0.8	36.2	35.1	1.32
160			30.1	1.7	34.7	34.6	0.12
161	Porbander	..	15.7	0.5	37.9	36.8	1.32
162			17.4	1.1	39.0	38.2	2.0
163			17.3	0.5	37.5	36.0	1.8
164			20.5	2.1	36.8	35.3	1.8
165			29.7	2.4	36.3	34.6	2.0
166			25.9	1.7	30.6	29.8	0.96
167			20.4	0.6	33.5	32.0	1.8
168			20.5	2.0	36.8	35.9	1.08
169	Bombay (W. I. G. Co., Ltd.)	..	32.9	1.5	27.5	26.8	0.84
170			30.1	4.1	30.7	29.2	1.8
171			37.7	2.1	30.7	29.6	1.32
172			28.3	2.7	28.8	28.0	0.96
173			27.6	2.3	30.2	29.2	1.2
174			28.3	2.7	28.8	27.0	2.0

TABLE II—*contd.*

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
175	Nepalgarh	..	18.5	1.1	45.1	44.1	1.2
176			21.2	1.3	44.6	42.9	2.0
177			26.6	1.2	40.2	39.2	1.2
178			20.6	1.5	44.1	43.0	1.32
179			21.7	1.1	43.8	42.1	2.0
180			20.6	2.7	41.0	40.1	1.08
181			20.8	2.4	43.0	42.0	1.2
182			17.8	2.1	41.9	40.3	1.92
183			16.9	1.8	43.6	42.1	1.8
184			17.6	1.7	45.0	43.6	1.68
185	N. W. F. P.	..	26.6	3.0	31.0	30.0	1.2
186			22.8	2.6	33.7	32.4	1.56
187			26.5	3.1	35.2	34.1	1.32
188			28.1	3.2	35.5	34.1	1.68
189			24.2	2.5	34.7	33.0	2.0
190			25.8	3.1	36.8	36.0	0.96
191			20.3	2.6	35.5	34.1	1.68
192			21.6	2.6	34.3	33.0	1.56
193			24.2	2.8	37.8	36.0	2.0
194	Jhallar (C. P.)	..	25.1	3.0	30.8	30.1	2.0
195			27.4	1.2	32.7	31.0	2.0
196			17.0	1.0	34.5	33.2	1.56
197			17.8	1.1	34.5	33.2	1.56
198			21.6	1.0	28.0	26.9	1.32
199			18.0	1.5	36.0	35.0	1.2
200			23.8	3.0	26.8	25.0	2.0
201			23.1	2.3	31.4	30.0	1.68
202			21.9	1.0	25.1	24.0	1.32
203	Mandla (C. P.)	..	22.4	0.7	32.7	31.3	1.68

TABLE II—*contd.*

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
204	Mandla— <i>contd.</i>		22.1	1.9	34.1	32.7	1.68
205			23.8	0.8	37.6	36.0	1.92
206			25.4	1.2	34.6	34.0	0.72
207			22.5	2.2	33.8	32.1	2.0
208			22.4	2.2	33.9	32.7	1.44
209			21.6	3.0	35.7	34.2	1.8
210			21.8	1.0	25.0	24.1	1.08
211			32.0	2.0	29.2	28.3	1.08
212			32.6	2.0	29.2	29.0	0.24
213	Nadiad (Bombay)	..	25.7	1.5	27.7	26.9	0.96
214			25.6	1.4	27.4	26.5	1.08
215			31.2	1.7	29.9	29.8	0.12
216			31.0	1.7	30.0	29.2	0.96
217			29.1	1.8	28.8	27.9	1.08
218			36.4	1.7	29.4	28.7	0.84
219			36.2	1.4	29.5	28.6	1.08
220			28.3	2.4	28.8	27.3	1.8
221			28.0	2.1	28.6	26.9	2.0
222			27.6	2.3	30.2	28.8	1.68
223			30.6	2.0	28.6	26.9	2.0
224			30.8	2.1	28.7	27.4	1.56
225			32.7	1.4	27.5	26.8	0.84
226			32.9	1.5	27.6	26.9	0.84
227			39.1	4.0	25.7	24.8	1.08
228			37.7	2.1	30.7	29.3	1.68
229			29.3	1.1	31.1	30.1	1.2
230			31.9	1.5	29.2	28.6	0.72
231	Amraoti (C. P.)	..	17.8	0.6	33.6	32.6	1.2
232			20.2	1.0	31.9	30.8	1.32

TABLE II—*contd.*

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
233	Amraoti— <i>contd.</i>		27.9	0.7	28.3	26.7	1.02
234			25.6	1.7	30.8	36.0	0.96
235			30.8	0.7	32.3	31.6	0.84
236			28.6	2.1	36.2	35.9	0.60
237			22.9	1.7	41.1	40.1	1.2
238			20.5	2.3	37.3	36.4	1.08
239			28.3	2.7	25.9	24.9	1.2
240			18.5	2.0	38.8	39.9	1.08
241	Tikoti (C. P.)	..	28.3	1.1	30.8	30.0	0.96
242			27.2	1.0	29.8	28.9	1.08
243			27.3	1.0	29.8	28.9	1.08
244			28.8	1.4	20.3	28.4	1.08
245			21.8	0.8	42.0	41.6	1.2
246			28.3	1.5	32.8	31.8	1.2
247			22.8	0.9	41.2	40.3	1.08
248			20.8	1.3	39.8	38.8	1.2
249	Barmer (Jodhpur)	..	17.6	0.8	34.9	33.8	1.32
250			22.4	1.5	33.6	32.7	1.08
251			22.6	1.7	33.8	32.7	1.32
252			22.9	1.3	36.0	34.8	1.44
253			16.3	1.8	36.1	35.0	1.32
254			16.8	1.8	35.0	34.1	1.08
255			17.8	0.6	33.6	32.7	1.08
256			20.0	1.0	31.9	30.9	1.2
257	Jallar (C. P.)	..	27.7	0.7	28.2	26.9	1.56
258			27.9	0.9	28.3	27.4	1.08
259			25.6	1.0	36.7	35.9	0.96
260			30.6	0.7	32.3	31.0	1.56
261			28.6	2.0	36.0	34.9	1.32

TABLE II —*contd.*

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
262	Jallar (C. P.)— <i>contd.</i>	..	28.3	2.4	37.2	36.7	0.60
263	Jhansi
264			21.5	1.4	29.4	28.3	1.32
265			21.6	1.1	34.0	32.4	0.72
266			28.5	1.9	37.0	36.4	0.72
267			26.8	1.4	37.8	36.8	1.2
268			24.5	1.8	39.4	38.5	1.08
269	Porbander	..	24.7	2.5	39.7	38.8	1.08
270			17.4	1.1	39.9	38.8	1.32
271			17.0	0.5	37.5	36.4	1.32
272			15.6	0.0	35.7	34.6	1.32
273			14.5	1.1	37.8	36.8	1.2
274			2.50	2.1	36.8	35.9	1.08
275			1.95	0.6	34.0	33.0	1.2
276			29.7	2.2	36.8	35.9	1.08
277	Akola (C. P.)	..	22.3	0.8	35.8	31.8	1.2
278			22.0	2.0	34.2	33.3	1.08
279			23.9	0.9	37.7	36.7	1.2
280			28.5	1.2	34.7	33.8	1.08
281			24.4	2.0	32.3	31.3	1.2
282			21.7	3.3	35.7	34.6	1.32
283	Gonda (U. P.)	..	18.4	1.1	37.7	36.6	1.2
284			26.2	2.8	36.2	35.3	1.32
285			25.8	1.7	36.9	36.0	1.08
286			19.6	2.2	36.9	35.9	1.2
287			19.8	1.6	36.1	34.8	1.58
288			24.6	3.2	37.5	36.8	0.84
289			20.0	2.0	38.0	37.0	1.2
290			25.0	2.9	33.5	32.4	1.32

TABLE II—*contd.*

Serial No.	Sample	Month	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
291	Gonda (U.P.)— <i>contd.</i>	..	24.4	3.0	36.8	35.8	1.2
292			18.2	1.0	35.5	36.6	1.08
293			26.0	2.0	36.2	35.2	1.2
294	Yectimal (C. P.)	..	30.5	1.9	28.7	27.7	1.2
295			31.0	1.2	30.0	29.1	1.08
296			28.2	1.2	32.1	32.0	0.12
297			23.4	1.0	38.4	37.45	1.14
298			31.6	1.4	34.5	33.40	1.32
299			31.4	1.0	33.5	32.60	1.08.

Samples from the Central Control Laboratory, Cawnpore

Serial No.	Sample	Month	Acid value	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
300	Gonda	..	8.0	17.4	1.1	34.4	34.0	0.48
301			9.0	22.3	1.14	29.4	27.4	2.4
302			9.6	20.8	1.9	19.5	17.5	2.4
303			10.1	18.9	3.0	28.3	26.0	2.76
304			11.0	14.5	1.1	20.6	17.4	2.64
305			11.4	19.7	1.7	26.0	24.3	2.16
306			11.5	20.8	1.7	27.1	25.0	2.52
307			12.1	22.3	1.3	19.4	17.0	2.88
308	Aligarh	..	13.0	23.0	1.8	21.0	18.4	3.12
309			6.1	25.1	1.2	28.9	26.9	2.4
310			7.0	24.3	1.4	27.0	25.1	2.2
311			7.1	24.1	1.6	26.0	24.0	2.4
312			7.5	26.0	1.6	24.8	22.6	2.6
313			7.1	26.0	1.7	23.0	21.0	2.4
314			7.0	27.0	1.8	26.9	24.8	2.6
315			7.4	26.9	1.6	27.0	25.0	2.4

TABLE II—*contd.*

Serial No.	Sample	Month	Acid value	R.M.	P.V.	I.V.	SCN.V.	Per cent of linoleic acid
316	Shikohabad	..	3.4	34.0	2.1	34.6	34.0	0.7
317			4.0	36.2	1.9	34.0	32.0	2.4
318			3.2	23.0	1.7	41.0	40.0	1.2
319			2.0	20.4	1.7	41.0	38.8	1.4
320			2.1	27.9	2.4	26.1	24.0	2.5
321			1.9	18.5	2.0	38.8	37.0	2.1
322			4.1	20.4	2.1	32.0	30.0	2.4
323			4.0	21.8	2.3	34.0	32.0	2.4
324			3.0	22.9	1.7	33.0	30.8	2.6
325			3.2	22.0	1.8	26.8	24.7	2.5
326	Ghee Grading Station (Goya).	..	10.5	15.7	1.1	30.8	28.7	2.5
327			10.0	8.9	2.0	34.0	32.0	2.4
328			10.1	20.3	2.2	36.0	34.1	2.3
329			10.8	22.1	2.5	30.1	28.0	2.5
330			11.0	22.0	2.4	29.4	27.0	2.8
331	187 B. J.	..	7.6	22.8	2.6	30.0	28.0	2.4
332	188 B. J.	..	7.6	21.0	2.4	28.4	26.2	2.6
333	189	..	7.8	21.0	2.4	27.4	25.1	2.7
334	190	..	7.85	23.0	1.8	31.0	29.0	2.4
335	191	..	9.0	22.1	1.7	29.4	27.5	2.3
336	192	..	8.2	24.0	1.9	26.0	24.1	2.3
337			9.5	23.1	1.6	27.0	24.8	2.6
338	180	..	4.0	27.0	1.2	32.0	30.0	2.4
339	181	..	10.5	32.2	1.6	26.7	26.0	0.8
340	182	..	12.1	30.1	1.8	28.1	26.0	2.5
341	183	..	8.6	27.9	1.9	37.8	25.8	2.4
342	184	..	8.5	28.3	2.0	26.0	23.8	2.6
343	185	..	6.5	23.2	1.6	36.1	34.0	2.5

TABLE II—*concl'd.*

Serial No.	Sample	Month	Acid value	R.M.	P.V.	L.V.	SCN.V.	Per cent of linoleic acid
344	186	..	6.1	24.0	1.6	36.5	34.0	3.0
345	187	..	7.9	27.0	1.8	29.8	28.0	2.2
346	188	..	7.6	25.6	1.9	26.0	24.0	2.4
347	189	..	7.9	26.0	1.7	31.0	28.7	2.7
348	190	..	8.7	24.0	1.8	29.0	27.0	2.4
349	191	..	9.9	25.1	1.6	26.0	24.0	2.4
350	192	..	13.0	24.1	1.7	27.0	24.8	2.7
351	Gonda	..	0.1	19.8	2.2	30.0	35.9	1.2
352				18.4	1.1	37.7	36.4	1.5
353				26.2	2.1	36.2	35.0	1.4
354				24.6	2.5	38.0	36.8	1.4
355				20.0	3.5	38.0	37.0	1.2
356				18.2	1.0	37.5	36.0	1.8
357				25.8	1.9	36.0	34.8	1.4
358				20.0	2.0	33.5	32.4	1.3

Three of the samples were analyzed for component acids by the ester fractionation method with an electrically heated and packed column using the method employed by Smith and Dastur [1938] of direct methanolysis and separation of the lower acids in some quantity before undertaking the Twitchell separation.

The results of ester fractionation are given in Table III (p. 317).

DISCUSSION AND SUMMARY

It will be seen from the results that the main proposition is confirmed, that is genuine *ghee* has not got more than two per cent of linoleic acid. Therefore, a safer criterion for detection of adulteration of *ghee* of low R. M. value will be to determine its correlation constants [Achaya and Banerjee, 1947] and also its linoleic acid content, *i.e.* unsaturated acids (iodine and thiocyanogen values). If the difference is not above two per cent it can be passed as genuine. This is a much easier and simpler test than phytosterol acetate test.

A closer examination of the data shows that while by the ester fractionation method, the linoleic acid is well below 2 per cent, 10 per cent of the results of iodine and thiocyanogen value determinations give figures between 2 to 2.5 per cent. An

TABLE III

Characteristics of the samples of ghee analyzed by ester fractionation

Origin	Wardha (C. P.)	Ahmedabad	Junagadh
General characteristics	Excellent flavour and texture, quite white in colour	Good flavour light yellow in colour	Good flavour, small grains and pale yellow
R. M.	18.5	30.1	36.0
P. V.	0.4	0.7	1.0
I. V.	32.0	38.5	37.0
S. V.	238.0	228.0	225.0
Refractive index at 40°C.	1.456	1.4564	1.4562
Acidity as oleic acid	0.04	0.03	0.07

Fatty acid composition of ghees analyzed by ester fractionation

Acid	Wardha (C. P.)	Ahmedabad	Junagadh
<i>Saturated</i>			
Butyric	11.55	3.88	9.2
Caproic	0.21	0.64	2.8
Caprylic	0.8	0.98	2.7
Capric	2.3	1.85	3.5
Lauric	2.4	2.56	5.2
Myristic	12.3	11.80	14.8
Palmitic	26.5	23.46	27.2
Stearic	10.1	11.53	8.51
Arachidic	..	1.08	1.2
<i>Unsaturated</i>			
Decenoic	0.2	0.24	0.3
Dodecenoic	0.14	0.24	0.2
Tetradecenoic	1.0	0.94	1.5
Palmitoleic	2.9	3.15	5.2
Oleic	27.0	3.557	15.3
Linoleic	1.80	1.17	1.90
Gadoleic	0.70	0.82	0.70

N

explanation has to be found for this deviation from the proposition. Two other investigations carried on by B. N. Banerjee and K. T. Achaya (unpublished data) provided the necessary explanation. If the component acids of the free fatty acids of a stored or rancid high acid *ghee* or edible oil is analyzed, then it is found that as a result of the acidity development, acids of a different nature, often by carbon breakdown of normal acids, are formed and this happens with both saturated and unsaturated acids. Again, fresh *ghee* gives only a greenish fluorescence when viewed in ultra-violet light with a yellow colour depending on the carotene content. But if the *ghee* is bleached with carbon to remove the carotene, then only the greenish fluorescence persists. Now, if a stored or rancid *ghee* with high f.f.a. is fluoresced, then a bluish colour appears [Achaya and Banerjee, 1945]. Therefore a number of samples that gave more than two per cent linoleic acid was examined for their free fatty acidity and fluorescence colour in ultra-violet light. The results are given in Table IV.

TABLE IV

Table showing linoleic acid content, f.f.a. and fluorescence in ultra-violet light

Sample (10 months old)	f.f.a.	Linoleic acid content	Fluorescence in ultra-violet light
Shikohabad	2.0	2.5	Pale yellow with bluish tint
	3.2	2.5	Pale blue with tint of green
	4.0	2.4	Bluish purple
Aligarh	6.1	2.4	Pale blue
	7.0	2.4	Pale blue
	7.5	2.6	Bluish violet
Gonda	8.0	2.4	Pale blue with faint green tint
	9.0	2.4	Pale blue
	9.6	2.4	Pale blue
Cawnpore	10.5	2.5	Pale blue with touch of green
	10.8	2.5	Pale blue
	11.0	2.8	Light blue

TABLE IV—*contd.*

Sample (10 months old)	f.f.a.	Linoleic acid content	Fluorescence in ultra-violet light
Bangalore (I. D. R. I.)			
Date of preparation	Date of analysis 14-8-48		
8-8-48	0.05	1.82	Greenish with a tint of yellow
4-6-47	1.06	2.4	Pale green with blue tint
11-7-47	0.50	2.4	Pale yellow with tinge of green
17-7-47	0.44	2.30	Pale yellow
2-8-47	0.43	2.30	Light green
14-8-47	0.40	2.0	Greenish yellow
1-9-47	0.28	1.8	Yellowish green
13-9-47	0.23	1.7	Greenish
16-10-47	0.16	1.70	Green
14-11-47	0.16	1.60	Pale green
12-12-47	0.12	1.70	Light green
4-1-48	0.08	1.50	Yellowish green
7-2-48	0.05	1.50	Light green
12-3-47	0.04	1.40	Light green with yellow tint

The free fatty acidity, length of storage of *ghee* and the bluish fluorescence confirm the proposition with additional data that in genuine fresh *ghee*, the linoleic acid content is never more than two per cent. In those *ghees* where the f.f.a. is high or rancidity is developed or stored for some time with development of off-flavour, the apparent linoleic acid (calculated from the iodine and thiocyanogen values) goes up from more than 2 per cent to 2.5 per cent. Though the difference between iodine and thiocyanogen values is expressed as linoleic acid, for some unknown reason probably the production of other acids containing two double bonds by some sort of desaturation mechanism involving mono-ethenoid unsaturated acids is quite possible. The present investigation provides an explanation for the non-linear relationship of blended or mixed *ghee* in the co-relationship of the constants of *ghee*. The investigation now presented has given us sufficient data to state that the linoleic acid content of *ghee*, determined by calculation from iodine and thiocyanogen values, can be used as a means for distinguishing especially low R.M. genuine *ghee* from low R. M. *ghee* artificially brought about by adulteration. Caution must, however, be given against coconut oil type of adulteration which will easily be distinguished by Polenske data.

A simple iodine and thiocyanogen values determination does away with the necessity for phytosterol acetate test. It throws light on the possibility of fluorescence analysis of *ghee* to be used as a physical method for grading of the latter. If only filters could be found out to distinguish products of rancidity from adulterants of *ghee* then the process would be still more simplified and adulteration question solved beyond measure.

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ABSTRACT

THE EFFECT OF HEXACHLORETHANE-BENTONITE SUSPENSION ON THE LANCET FLUKE, *DICROCOELIUM LANCEOLATUM*MARTIN, M. KAPLAN AND SOTIRIOS, SAKELLARION (1947). *Vet. Med.* 42, pp. 23-24

LIVER flukes are very common and economically important parasites of ruminants. In the hilly tracts where land snails abound, mixed infections of *Fasciola* spp. and *Dicrocoelium dendriticum* (syn. *D. lanceolatum*) are found. While carbon tetrachloride is an effective drug against adult specimens of *Fasciola* spp., it is ineffective against *D. dendriticum* and immature *Fasciola* spp., and is contraindicated under certain conditions. In this paper the joint authors report the results of their investigations on the efficacy of hexachlorethane against the lancet fluke in sheep. Hexachlorethane crystals were ground to a fine powder with a mortar and pestle. Bentonite was added to form a final concentration by weight of 90 per cent hexachlorethane and 10 per cent bentonite. Of that mixture 497 gm. were added to 710 c. c. of water giving a final volume of about 900 c. c. A dose of 30 c. c. of that suspension, which contained 15 gm. of hexachlorethane, was administered to each of the five sheep showing advanced symptoms of distomiasis. The animals were three to five years of age and weighed between 45 and 50 lb. Food was withheld 6 hours before and 15 hours after the administration of the suspension. The animals were slaughtered 92 hours later and a massive infection with *D. dendriticum* was found in the livers of four of them. Many young forms of *F. hepatica* were also present. The livers were not sclerotic. Hundreds of dead flukes were pressed out from the bisected bile ducts of each of the infected livers. No living flukes were found. The striking results obtained in that small scale experiment indicated the advisability of more comprehensive studies on the use of hexachlorethane in the treatment of *D. dendriticum* infections in ruminants. [H.D.S.].

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